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(57) Abstract

Methods are described for the identification and preparation of high-affinity nucleic acid ligands to human secretory phospholipase A_2(sPLA$_2$) and human immunodeficiency virus type-1 GAG (HIV-1 GAG). Included in the invention are specific RNA ligands to sPLA$_2$ and HIV-1 GAG identified by the SELEX method. Also included are high-affinity modified RNA ligands and ssDNA ligands to vascular endothelial growth factor (VEGF).

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HIGH-AFFINITY OLIGONUCLEOTIDE LIGANDS TO SECRETORY PHOSPHOLIPASE A2 (SPLA2)

FIELD OF THE INVENTION

Described herein are methods for identifying and preparing high-affinity nucleic acid ligands to secretory phospholipase A2 ($sPLA_2$) and human immunodeficiency type-1 GAG (HIV-1 GAG). The method utilized herein for identifying such nucleic acid ligands is called the SELEX process, an acronym for Systematic Evolution of Ligands by EXponential enrichment. This application also describes high-affinity nucleic acid ligands to VEGF, identified through the SELEX method. Specifically disclosed herein are high-affinity nucleic acid ligands to $sPLA_2$, VEGF, and HIV-1 GAG. The invention includes high-affinity RNA ligands that bind to $sPLA_2$ and inhibit the enzymatic activity of $sPLA_2$, modified RNA ligands and ssDNA ligands to VEGF, and RNA ligands to HIV-1 GAG. The oligonucleotides of the present invention are useful as pharmaceuticals or diagnostic agents.

BACKGROUND OF THE INVENTION

$sPLA_2$

Inflammation is a local response to tissue damage or foreign material which is designed to isolate and/or destroy injured tissues and foreign substances. Uncontrolled inflammatory responses may irreparably damage host tissue, as in the chronic inflammation of rheumatoid arthritis or lead to multiple organ failure and death as in the systemic inflammation of septic shock .

Eicosanoids are metabolic products of the essential fatty acid, arachadonic acid and are known to play a central role in the inflammatory response. Arachadonic acid is an integral component of cell membranes where it is commonly found as an sn-2 acyl or akyl ester of 3-sn-phosphoglycerides. Phospholipase A2 (PLA_2) is a class of enzymes that specifically catalyze the hydrolysis of the sn-2 acyl or alkyl ester of phosphoglycerides, producing equimolar quantities of lysophospholipids and free fatty

acids. The PLA_2 catalyzed hydrolysis of one such membrane phospholipid, alkyl-arachidonyl-glycerophosphatidylcholine, yields free arachidonic acid and lyso-platelet activating factor (lyso-PAF), the precursor of PAF, in equimolar amounts. Since the availability of free arachidonic acid is rate limiting for eicosanoid synthesis, the pro-inflammatory role of PLA_2 is thought to be the consequence of its role in arachadonic acid metabolism.

Three mammalian PLA_2 enzymes are known. Pancreatic PLA_2 is related to the type I PLA_2 from the venom of Elapidae and Hydrophide both in primary and tertiary structure. A digestive enzyme, synthesized as a proenzyme in the pancreas, it is unlikely to play a central role in inflammatory conditions.

Two non-pancreatic mammalian enzymes have been described. One is a high molecular weight intracellular enzyme. The other is a soluble enzyme, $sPLA_2$, and is of particular interest because it has been isolated from inflammatory exudates, such as synovial fluid (Seilhamer *et al.* (1989) J. Biol. Chem. 264:5335-5338) and from platelets (Krammer *et al.* (1989) J. Bio. Chem. 264:5768-5775). $sPLA_2$ is known by several equivalent names, including secretory phospholipase A_2, soluble phospholipase A_2 and synovial phospholipase A_2, all of which can be used interchangeably. This enzyme, which has been sequenced, has a molecular weight of 14 kD, a pI > 10 and is homologous to type II PLA_2 from the venoms of Crotalidae and Viperidae (Krammer *et al.* (1989) *supra*; Scott *et al.* (1991) Science 254:253-255; Wery *et al.* (1991) Nature 352:79-82).

The involvement of $sPLA_2$ in the inflammatory response is supported by two types of data. First, elevated levels of serum PLA_2 activity have been observed in diseases such as endotoxemia (Vadas and Hay (1983) Can. J. Physiol. Pharmacol. 61:561-566), sepsis (Vadas (1984) J. Lab. Clin. Med. 104:873-881; Nevalainen *et al.* (1992) Clin. Chem. 38:1824-1829), rheumatoid arthritis (Pruzanski *et al.* (1988) J. Rheumatol. 15:1351-1355), pancreatitis (Nevalainen *et al.* (1993) Gut 34:1133-1136) and uremia (Costello *et al.* (1990) Clin. Chem. 36:198-200) and in some studies correlate with severity and outcome. One of the best documented cases is acute pancreatitis in which $sPLA_2$ immuno-reactive activity, but not pancreatic PLA_2 immuno-reactive activity, is correlated with serum PLA_2 enzymatic activity. The duration of the elevated

levels is longer for the more severe necrotizing pancreatitis than the less severe oedmatous form (Nevalainen *et al.* (1993) *supra*).

Second, in both animal and tissue models (Snyder *et al.* (1993) J. Pharmacol. and Therapeutics 266:1147-1155), the introduction of $sPLA_2$ results in an inflammatory like response.

VEGF

Neovascularization or angiogenesis is the process in which sprouting new blood vessels are formed from the existing endothelium in response to external stimuli that signal inadequate blood supply. Angiogenesis is generally rare under normal physiological conditions but frequently accompanies certain pathological conditions such as psoriasis, rheumatoid arthritis, hemangioma, and solid tumor growth and metastasis (Folkman & Klagsbrun, 1987) Science 235:442-447; Kim *et al.*, (1993) Nature 362:841-844). Several growth factors that are capable of inducing angiogenesis *in vivo* have been identified to date including basic and acidic fibroblast growth factors (aFGF, bFGF), transforming growth factors α and β (TGFα, TGFβ), platelet derived growth factor (PDGF), angiogenin, platelet-derived endothelial cell growth factor (PD-ECGF), interleukin-8 (IL-8), and vascular endothelial growth factor (VEGF).

VEGF was originally purified from guinea pig ascites and tumor cell cultures as a factor that increases vascular permeability (Senger, D.R. *et al.* (1983), Science 219:983-985) and it has therefore also been referred to as vascular permeability factor (VPF). VEGF is a heat and acid-stable, disulfide-linked homodimer. Four isoforms have been described (121, 165, 189 and 206 amino acids, respectively) and are believed to be the result of alternative splicing of mRNA. Despite the presence of an identical N-terminal hydrophobic signal sequence in all molecular isoforms of VEGF, only the two lower molecular weight species are efficiently secreted (Ferrara, N. *et al.* (1991) J. Cell. Biochem. 47:211-218). The predominant VEGF isoform in most cells and tissues is the 165 amino acid species. Although VEGF is typically glycosylated, glycosylation is only required for efficient secretion but not for activity (Yeo, T-.K. *et al.* (1991) Biochem. Biophys. Res. Commun. 179:1568-1575; Peretz, D. *et al.* (1992) Biochem. Biophys. Res. Commun. 182:1340-1347). The amino acid sequence of VEGF is highly conserved across species and exhibits a modest but significant homology (18-20%) to PDGF A and

B (Jakeman L.B. *et al.* (1992) J. Clin. Invest. 89:244-253; Ferrara *et al.* (1992) Endocrine Rev. 13:18-32).

Unlike other angiogenic growth factors, target cell specificity of VEGF is limited to vascular endothelial cells. The biological actions of VEGF are mediated through its interaction with specific cell-associated receptors which have been identified in the majority of tissues and organs (Jakeman, L.B. (1992) J. Clin. Invest. 89:244-253). Three high-affinity receptors for VEGF have been cloned to date: flt1, kdr/flk-1 and flt4 (Vaisman, N. *et al.* (1990) J. Biol. Chem. 265:19461-19466; de Vries, C. *et al.* (1992) Science 255:989-991; Galland, F. *et al.* (1993) Oncogene 8:1233-1240). These receptors belong to a family of transmembrane tyrosine kinases and bind VEGF with dissociation constants between 10^{-11} M to 10^{-12} M. Recent experiments have shown that binding of VEGF to its high-affinity receptors is significantly enhanced by heparin or cell surface-associated heparin-like molecules (Gitay-Goren, H. (1992) J. Biol. Chem. 267:6093-6098).

In addition to promoting the growth of vascular endothelial cells and inducing vascular leakage, VEGF is also known to induce the proteolytic enzymes interstitial collagenase, urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) (Unemori E. *et al.* (1993) J. Biol. Chem. in press; Pepper, M.S. *et al.* (1992) Biochem. Biophys. Res. Commun. 181:902-). These enzymes are known to play a prominent role in angiogenesis-related extracellular matrix degradation.

As a secreted and specific mitogen for endothelial cells, VEGF may be one of the major angiogenesis inducers *in vivo*. Several recent observations have supported this notion. For example, the expression of VEGF and its receptors accompanies angiogenesis associated with (i) embryonic development (Breier, G. *et al.* (1992) Development 114:521-532), (ii) hormonally-regulated reproductive cycle and (iii) tumor growth (Dvorak, H.F. (1991) J. Exp. Med. 174:1275-1278; Shweiki, D. *et al.* (1992) Nature 359:843-845; Plate, K.H. *et al.* (1992) Nature 359:845-848). It is relevant to note that aggressive tumor growth is accompanied by the generation of necrotic areas where oxygen and nutrient supplies are inadequate. Oxygen deprivation (hypoxia) in tissues is a known angiogenesis stimulant. Interestingly, VEGF expression was found to be the highest in tumor cells facing the necrotic areas (Shweiki, D. *et al.* (1992) *supra*; Plate,

K.H. *et al.* (1992) *supra*). It has therefore been suggested by these authors that VEGF plays a key role in hypoxia-induced angiogenesis.

Recent experiments with neutralizing monoclonal antibodies (MAbs) to VEGF have been especially meaningful for establishing that this growth factor is an important tumor angiogenesis inducer *in vivo* (Kim, K.J. *et al.* (1993) Nature 362:841-844). Immunocompromized (nude) mice injected with human rhabdomyosarcoma, glioblastoma or leiomyosarcoma cell lines rapidly develop tumors. Specific neutralizing MAb to VEGF were found to inhibit the growth of these tumors. The density of tumor vasculature was decreased in MAb-treated animals as compared to controls. The same MAb, on the other hand, had no effect on the growth rate of the tumor cells *in vitro* suggesting that the growth inhibition was not mediated at the cellular level and appears to be mediated by the 165-amino acid isoform of VEGF.

HIV-1 GAG

The type-1 human immunodeficiency virus (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS) (Levy (1993) Microbiol. Rev. 57:183-289). There are estimated to be about 1 million people infected with HIV-1 in the United States and over 13 million worldwide. In some localities over 80% of the population is infected. The average time between HIV-1 infection and death is about ten years, making the course of treatment long and expensive. There is no cure for HIV-1 infection. Five mononucleoside drugs (AZT, ddI, ddC, d4T and 3TC) have been approved for use in the United States for the treatment of HIV-1 infection. Each of these drugs inhibits the HIV-1 reverse transcriptase protein. In addition, a drug (Invirase) that inhibits the HIV-1 protease has been approved for clinical use. However, the usefulness of these drugs is limited by the rapid emergence of drug resistance mutants (Larder (1989) Science 246:1155-1158; Ridky and Leis (1996) J.Biol.Chem. 270:29621-29623). More effective treatments or a cure for HIV-1 infection would be highly desirable. As a result, other HIV-1 proteins are being explored as possible drug targets.

The HIV-1 gag (group specific antigen; also called assemblin) protein is a polyprotein composed of several HIV-1 proteins including matrix, capsid, p1, p2, p6, and nucleocapsid. Gag is myristylated on the N-terminus. Myristylation is required for localization of gag to inner surface of plasma membrane. Once gag accumulates to a

high enough concentration at the membrane it begins to multimerize. Multimerization of gag results in the formation of an immature virion that is released from the cell. After release of the virion, gag is processed into individual proteins by the HIV-1 protease leading to the formation of what is referred to as the mature virion. From electron microscopy studies, gag appears to be a cylindrical -shaped protein that is 85 Å long and 34 Å wide (Nurmut *et al.* (1994) Virology 198:288-296). There are estimated to be about 2000 gag proteins per virus.

The HIV-1 gag protein has multiple functions in the life cycle of HIV-1 that are reflective of the functions of its individual protein components (Gorelick and Henderson (1994) In: Human retroviruses and AIDS 1994: A compilation and analysis of nucleic acid and amino acid sequences. (Myers *et al.* eds.) Los Alamos Natl. Lab., Los Alamos, NM, pp.III-2 to III-10). Gag contains the structural proteins of HIV-1 and most of the functions of gag revolve around packaging of the viral RNA and assembly of the virus.

The matrix protein is a multifunctional protein that functions both during viral assembly and after infection. During assembly, along with the capsid protein, it is thought to mediate protein/protein interactions that direct the assembly and multimerization of the "core" of HIV-1. The NMR structure of matrix and the crystal structure of Simian Immunodeficiency Virus (SIV) matrix are known. The NMR structure of HIV-1 matrix shows that it resembles that of gamma interferon (Matthews (1994) Nature 370:666-668).

From the crystal structure of matrix, it was concluded that it forms trimers as intermediates in the assembly of HIV. Amino acids that may be involved in the interface between adjacent molecules were also identified. Matrix also has a region that forms a loop on the protein surface which is required for incorporation of the envelope protein into assembling viruses (Freed and Martin (1995) J.Virol. 69: 1984-1989; Freed and Martin (1996) J.Virol. 70: 341-51). If the envelope binding function of matrix were inhibited, then viruses without envelope proteins would result and these would be noninfectious.

After infection, matrix is required for entry of viral DNA into the nucleus prior to integration (Buckrinsky *et al.* (1993); Nature 365: 666-669). Tyrosine phosphorylation of matrix allows it to bind to the HIV-1 integrase protein/viral DNA complex (Gallay *et*

al. (1995) Cell 83: 569-576). The lysine-rich nuclear localization signal of matrix. located between amino acids 18 and 28, then directs localization of the integration complex into the nucleus. The vpr protein can also mediate nuclear import of integration complexes, and it has been shown that deletion of both matrix and vpr is required to inhibit nuclear import. Therefore, a drug may need to inhibit the function of both vpr and matrix in order to affect nuclear import and integration.

The capsid protein ultimately forms the electron dense, cone-shaped core of HIV-1. Amino acids 240-430 are involved in dimerization of capsid. Capsid is phosphorylated on serine and threonine, but the function of this phosphorylation is unknown. Capsid binds stoichiometrically to cyclophilin A, a cellular protein, leading to its incorporation into mature HIV-1 particles (Luban *et al.* (1994) Cell 73:1067-1078; Thali *et al.* (1994) Nature 372:363-365; Franke *et al.* (1994) Nature 372:359-362). Amino acids between positions 180-300 and capsid are required for cyclophilin binding. Cyclophilin A is the target for the action of immunosuppressive drugs related to cyclosporin A. Cyclosporin A and non-immunosuppressive derivatives such as Sandoz NM811 prevent cyclophilin A incorporation into HIV-1 and severely reduce HIV infectivity (Steinkasserer *et al.* (1995) J. Virol. 69:814-824; Bartz *et al.* (1995) Proc. Natl.Acad.Sci USA 92: 5381-5385). Mutants of capsid that do not bind cyclophilin A are also noninfectious. Thus, an inhibitor of capsid could prevent viral assembly or infectivity.

The nucleocapsid protein packages HIV-1 RNA into assembling viruses. Both nucleocapsid and gag bind to a specific region called Ψ that is about 200 bases long and is located at the 5' end of the HIV-1 RNA (Aldovini and Young (1990) J. Virol. 64:1920-1926; Luban and Goff (1991) J. Virol. 65:3203-3212; Hayashi *et al.* (1992) Virology 188:590-599; Berkowitz *et al.* (1993) J. virol 67:7190-7200; Richardson *et al.* (1993) J. Virol. 67:3997-4005; Sakuguchi *et al.* (1993) Proc. Natl. Acad. Sci. USA 90:5219-5223). Two HIV-1 RNAs are packaged into each virus in a parallel orientation. Dimerization of the RNA is mediated by a "dimer linkage site" that is thought to be within or overlapping Ψ (Darlix *et al.* (1990) J. Mol. Biol. 216:689-699; Marquet *et al.* (1991) Nucl. Acid Res. 19:153-159; Awang and Sen (1993) Biochem. 32:11453-11457; Skripkin *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:4945-4949). It has been suggested

that HIV-1 RNA dimerization may involve guanine quartet structures (Sundquist and Heaphy (1993) Proc. Natl. Acad. Sci. USA 90:3393-3397). However, more recently strong evidence for the involvement of kissing loops in the binding of HIV RNA to gag has been obtained (Laughrea *et al.* (1994) Biochemistry 33:13464-13474; Paillart *et al.* (1994) J. Biol. Chem. 269:27486-27493; Skripkin *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:4945-4949). The RNA sequences within the Ψ region that are required for gag binding appear to overlap with those required for packaging of viral RNA (Luban and Goff (1994) J. Virol. 68:3784-3793), but it is not clear yet if sequences required for gag binding to RNA and packaging of the RNA into the virus are identical. Nucleocapsid binding to Ψ requires two zinc finger domains and a basic region (Dorfmann *et al.* (1993) J. Virol. 67:6159-6169; Dannull *et al.* (1994) EMBO J. 13:1525-1533). The structure of nucleocapsid has been determined by nuclear magnetic resonance spectroscopy (Morellet *et al.* (1994) J. Mol. Biol. 235:287-301).

The nucleocapsid protein has nucleic acid annealing and melting activities which are thought to be required at several points in the viral life cycle such as (1) melting of the $tRNA_{lys,3}$ reverse transcription primer and annealing to the viral RNA, (2) melting and reannealing of viral DNA to viral RNA at various "jumping" steps of reverse transcription, and (3) melting and annealing of two genomic RNAs that are subsequently packaging together into assembling virions (Darlix, *et al.* (1995) J.Mol.Biol. 254:523-537). Disruption of events (1) or (2) by nucleic acid ligands would inhibit reverse transcription and prevent provirus formation. Disruption of event (3) would lead to viral particles that would lack viral RNA. While such particles would be "infectious" in the sense they would be able to bind and fuse to cells, they would not result in the production of new virions within those cells.

The p6 protein may play a role in budding of viruses from cells since mutants that lack p6 are defective in budding (Gottlinger *et al.* (1991) Proc. Natl. Acad. Sci. USA 88:3195-3199). The p6 protein also binds the HIV-1 vpr protein leading to its stoichiometric incorporation into viruses (Paxton *et al.* (1993) J. Virol. 67:7229-7237). Vpr is involved in the nuclear localization of viral DNA prior to chromosomal integration. Vpr lacks a classical nuclear localization signal, so it has not been clear how it becomes localized in the nucleus. However, recently it has been reported that vpr may

bind to the glucocorticoid receptor which can translocate to the nucleus (Refaeli *et al.* (1995) Proc. Natl. Acad. Sci. USA 92:3621-3625). Vpr, along with matrix, is required for import of the integration complex. If a drug can inhibit both vpr and matrix functions in this process, then integration and production of new viruses would be prevented. Vpr has also been demonstrated to influence cellular differentiation and physiology, perhaps to favor HIV latency or replication (Levy *et al.* (1993) Cell 72:541-550; Levy *et al.* (1994) Proc. Natl. Acad. Sci. USA 91:10873-10877). Drugs that inhibit the vpr binding function of p6 would clearly be beneficial.

The peptides p1, p2 are cleaved from gag during its proteolytic processing and have been proposed to regulate the proteolysis of gag (Pettit *et al.* (1994) J. Virol. 68:8017-8027; Krausslich *et al.* (1995) J. Virol. 69:3407-3419), but they may also have other functions as well. Thus, nucleic acids that interact with p1 or p2 may disrupt the proteolytic processing of gag. This would lead to "immature" virions, which could be noninfectious.

In addition to gag, a gag-pol polyprotein precursor is encoded by the HIV genome. The pol gene encodes the enzymes of HIV-1 such as protease, integrase, reverse transcriptase, RNAse H, and integrase. Each of these proteins plays an indispensable role in the viral life cycle and inhibition of any of them would prevent HIV-1 replication. A ligand that binds to gag could inhibit the function of these proteins or their assembly into virions.

Gag is also post-translationally modified. Binding of a drug to gag could inhibit the formation of these modifications. As mentioned above, the matrix protein is phosphorylated on tyrosine, and it has been proposed that this allows matrix to bind to the integration complex of HIV-1 (Gallay *et al.* (1995) Cell 83: 569-576) in order to transport it into the nucleus. Blockage of this tyrosine phosphorylation might inhibit integration. Since vpr can also fulfill this function, inhibition of the phosphorylation of matrix alone may only slow the production of HIV-1 within an infected cell. Gag is also myristylated on the amino terminus of matrix. This modification is important for the localization of gag in the plasma membrane which leads to multimerization of gag and assembly of the core structure of HIV-1. Blockage of myristylation would prevent viral

assembly. The capsid protein is also phosphorylated but the function of this modification is not known.

THE SELEX PROCESS

A method for the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules has been developed. This method, Systematic Evolution of Ligands by EXponential enrichment, termed the SELEX process, is described in United States Patent Application Serial No. 07/536,428, entitled "Systematic Evolution of Ligands by Exponential Enrichment," now abandoned, United States Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled "Nucleic Acid Ligands," now United States Patent No. 5,475,096, United States Patent Application Serial No. 07/931,473, filed August 17, 1992, entitled "Nucleic Acid Ligands," now United States Patent No. 5,270,163 (see also PCT/US91/04078), each of which is herein specifically incorporated by reference. Each of these applications, collectively referred to herein as the SELEX Patent Applications, describes a fundamentally novel method for making a nucleic acid ligand to any desired target molecule.

The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of binding affinity and selectivity. Starting from a mixture of nucleic acids, preferably comprising a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding, partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules, dissociating the nucleic acid-target complexes, amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific, high affinity nucleic acid ligands to the target molecule.

The basic SELEX method has been modified to achieve a number of specific objectives. For example, United States Patent Application Serial No. 07/960,093, filed October 14, 1992, entitled "Method for Selecting Nucleic Acids on the Basis of Structure," describes the use of the SELEX process in conjunction with gel

electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. United States Patent Application Serial No. 08/123,935, filed September 17, 1993, entitled "Photoselection of Nucleic Acid Ligands" describes a SELEX-based method for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoinactivating a target molecule. United States Patent Application Serial No. 08/134,028, filed October 7, 1993, entitled "High-Affinity Nucleic Acid Ligands That Discriminate Between Theophylline and Caffeine," describes a method for identifying highly specific nucleic acid ligands able to discriminate between closely related molecules, termed Counter-SELEX. United States Patent Application Serial No. 08/143,564, filed October 25, 1993, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Solution SELEX," describes a SELEX-based method which achieves highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. United States Patent Application Serial No. 07/964,624, filed October 21, 1992, entitled "Methods of Producing Nucleic Acid Ligands" describes methods for obtaining improved nucleic acid ligands after the SELEX process has been performed. United States Patent Application Serial No. 08/400,440, filed March 8, 1995, entitled "Systematic Evolution of Ligands by EXponential Enrichment: Chemi-SELEX," describes methods for covalently linking a ligand to its target.

The SELEX method encompasses the identification of high-affinity nucleic acid ligands containing modified nucleotides conferring improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Examples of such modifications include chemical substitutions at the ribose and/or phosphate and/or base positions. SELEX-identified nucleic acid ligands containing modified nucleotides are described in United States Patent Application Serial No. 08/117,991, filed September 8, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," that describes oligonucleotides containing nucleotide derivatives chemically modified at the 5- and 2'-positions of pyrimidines. United States Patent Application Serial No. 08/134,028, *supra*, describes highly specific nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH_2), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe). United States Patent Application Serial No. 08/264,029,

filed June 22, 1994, entitled "Novel Method of Preparation of 2' Modified Pyrimidine Intramolecular Nucleophilic Displacement," describes oligonucleotides containing various 2'-modified pyrimidines.

The SELEX method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in United States Patent Application Serial No. 08/284,063, filed August 2, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Chimeric SELEX" and United States Patent Application Serial No. 08/234,997, filed April 28, 1994, entitled "Systematic Evolution of Ligands by Exponential Enrichment: Blended SELEX," respectively. These applications allow the combination of the broad array of shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable properties of other molecules. Each of the above-described patent applications which describe modifications of the basic SELEX procedure are specifically incorporated by reference herein in their entirety.

The isolation of specific antagonists to $sPLA_2$ would have multiple uses. First, $sPLA_2$ ligands would provide a useful tool for defining the enzyme's role in inflammatory responses and in diagnosing various inflammatory conditions. Second, $sPLA_2$ antagonists would be useful as an anti-inflammatory therapeutic. Given the cationic nature of $sPLA_2$ and the specificity of high affinity oligonucleotide ligands, SELEX technology is well suited for the isolation of $sPLA_2$ antagonists which would not cross react with pancreatic or high molecular weight PLA_2s. The present invention demonstrates the successful isolation of high-affinity oligonucleotide antagonists to $sPLA_2$.

The development of high affinity ligands of VEGF would be useful in the treatment of angiogenesis-mediated diseases. Herein described are high affinity nucleic acid ligands of VEGF.

SELEX ligands that bind to HIV-1 proteins provide another method of "intracellular immunization" (Baltimore (1988) Nature 335:395-396) against HIV-1 that differs from other nucleic acid-based technologies such as antisense (Zamecnik *et al.* (19°6) Proc. Natl. Acad. Sci. USA 83:4143-4146; Chatterjee *et al.* (1992) Science 258:1485-1488; Lisziewicz *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:11209-11213),

decoys (Sullenger *et al.* (1990) Cell 63:601-608), and ribozymes (Sarver *et al.* (1990) Science 247:1222-1225; Rossi *et al.* (1992) AIDS Res. Human Retroviruses 8:183-189), which have also been shown to have potential for being effective in preventing HIV-1 replication. In previous work, RNA molecules have been evolved that bind to the HIV-1 rev, tat, reverse transcriptase, and integrase proteins. Here the SELEX method is used to derive RNA molecules that bind to the HIV-1 gag polyprotein.

Gag is an attractive target for the development of SELEX ligands for several reasons. First gag is an RNA binding protein, which facilitates the *in vitro* evolution process. Second gag is found in the cytoplasm of a cell unlike its protein components (e.g., nucleocapsid) that are only found after viral release from the cell. This feature is an attractive one with regard to achieving intracellular immunization.

BRIEF SUMMARY OF THE INVENTION

The present invention includes methods of identifying and producing nucleic acid ligands to secretory phospholipase A2 ($sPLA_2$) and Human Immunodeficiency Virus type-1 gag polyprotein (HIV-1 GAG) and homologous polyproteins, and the nucleic acid ligands so identified and produced. For the purpose of this application, HIV-1 GAG refers to the polyprotein or any of its component parts (i.e., matrix, capsid, p1, p2, p6, and nucleocapsid). RNA sequences are provided that are capable of binding specifically to $sPLA_2$ and HIV-1 GAG. Specifically included in the invention are the RNA ligand sequences shown in Tables 2, 13, 15, 18, 19, and 20. Also herein described are high-affinity nucleic acid ligands to vascular endothelial growth factor (VEGF) identified through the SELEX method. Specifically included herein are modified RNA ligands to VEGF. Such modified RNA ligands may be prepared after the identification of 2'-OH RNA ligands or by performing the SELEX process using a candidate mixture of modified RNAs. In addition, such modified RNA ligands may be prepared from previously identified modified ligands. Post-SELEX modified RNA ligands are described herein and are provided in Table 7. Also included herein are ssDNA ligands to VEGF. The evolved ssDNA ligands are shown in Table 11.

Further included in this invention are methods of identifying nucleic acid ligands and nucleic acid ligand sequences to $sPLA_2$ or HIV-1 GAG comprising the steps of (a)

preparing a candidate mixture of nucleic acids, (b) partitioning between members of said candidate mixture on the basis of affinity to $sPLA_2$ or HIV-1 GAG, and (c) amplifying the selected molecules to yield a mixture of nucleic acids enriched for nucleic acid sequences with a relatively higher affinity for binding to $sPLA_2$ or HIV-1 GAG.

More specifically, the present invention includes the RNA ligands to $sPLA_2$ identified according to the above-described method, including those ligands listed in Table 2. Also included are RNA ligands to $sPLA_2$ that are substantially homologous to any of the given ligands and that have substantially the same ability to bind $sPLA_2$ and antagonize $sPLA_2$ activity. Further included in this invention are RNA ligands to $sPLA_2$ that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind $sPLA_2$ and antagonize $sPLA_2$ activity.

Additionally, the present invention includes the RNA ligands to HIV-1 GAG identified according to the above-described method, including those ligands shown in Tables 13, 15, 18, 19, and 20. Also included are RNA ligands to HIV-1 GAG that are substantially homologous to any of the given ligands and that have substantially the same ability to bind HIV-1 GAG and inhibit the function of HIV-1 GAG. Further included in this invention are nucleic acid ligands to HIV-1 GAG that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind HIV-1 GAG and inhibit the function of HIV-1 GAG.

The present invention also includes modified nucleotide sequences based on the RNA ligands identified herein and mixtures of the same.

Additionally, the present invention includes modified RNA ligands to VEGF shown in Table 7. Also included herein are ssDNA ligands to VEGF as shown in Table 11. Also included are ssDNA ligands to VEGF that are substantially homologous to any of the given ligands and that have substantially the same ability to bind VEGF and inhibit the function of VEGF. Further included in this invention are ssDNA ligands to VEGF that have substantially the same structural form as the ligands presented herein and that have substantially the same ability to bind VEGF and inhibit the function of VEGF.

DETAILED DESCRIPTION OF THE INVENTION

This application describes high-affinity nucleic acid ligands to $sPLA_2$, VEGF, and HIV-1 GAG, identified through the method known as the SELEX process. The SELEX process is described in U.S. Patent Application Serial No. 07/536,428, filed June 11, 1990, entitled "Systematic Evolution of Ligands by EXponential Enrichment," now abandoned, U.S. Patent Application Serial No. 07/714,131, filed June 10, 1991, entitled "Nucleic Acid Ligands," now United States Patent No. 5,475,096, United States Patent Application Serial No. 07/931,473, filed August 17, 1992, entitled "Nucleic Acid Ligands," now United States Patent No. 5,270,163, (see also PCT/US91/04078). These applications, each specifically incorporated herein by reference, are collectively called the SELEX Patent Applications.

In its most basic form, the SELEX process may be defined by the following series of steps:

1) A candidate mixture of nucleic acids of differing sequence is prepared. The candidate mixture generally includes regions of fixed sequences (i.e., each of the members of the candidate mixture contains the same sequences in the same location) and regions of randomized sequences. The fixed sequence regions are selected either: (a) to assist in the amplification steps described below, (b) to mimic a sequence known to bind to the target, or (c) to enhance the concentration of a given structural arrangement of the nucleic acids in the candidate mixture. The randomized sequences can be totally randomized (i.e., the probability of finding a base at any position being one in four) or only partially randomized (e.g., the probability of finding a base at any location can be selected at any level between 0 and 100 percent).

2) The candidate mixture is contacted with the selected target under conditions favorable for binding between the target and members of the candidate mixture. Under these circumstances, the interaction between the target and the nucleic acids of the candidate mixture can be considered as forming nucleic acid-target pairs between the target and those nucleic acids having the strongest affinity for the target.

3) The nucleic acids with the highest affinity for the target are partitioned from those nucleic acids with a lesser affinity to the target. Because only an extremely small number of sequences (and possibly only one molecule of nucleic acid) corresponding to

the highest affinity nucleic acids exist in the candidate mixture. it is generally desirable to set the partitioning criteria so that a significant amount of the nucleic acids in the candidate mixture (approximately 5-50%) are retained during partitioning.

4) Those nucleic acids selected during partitioning as having the relatively higher affinity to the target are then amplified to create a new candidate mixture that is enriched in nucleic acids having a relatively higher affinity for the target.

5) By repeating the partitioning and amplifying steps above, the newly formed candidate mixture contains fewer and fewer unique sequences, and the average degree of affinity of the nucleic acids to the target will generally increase. Taken to its extreme, the SELEX process will yield a candidate mixture containing one or a small number of unique nucleic acids representing those nucleic acids from the original candidate mixture having the highest affinity to the target molecule.

The SELEX Patent Applications describe and elaborate on this process in great detail. Included are targets that can be used in the process; methods for partitioning nucleic acids within a candidate mixture; and methods for amplifying partitioned nucleic acids to generate an enriched candidate mixture. The SELEX Patent Applications also describe ligands obtained to a number of target species, including both protein targets where the protein is and is not a nucleic acid binding protein.

The nucleic acid ligands described herein can be complexed with a lipophilic compound (e.g., cholesterol) or attached to or encapsulated in a complex comprised of lipophilic components (e.g., a liposome). The complexed nucleic acid ligands can enhance the cellular uptake of the nucleic acid ligands by a cell for delivery of the nucleic acid ligands to their intracellular target. U.S. Patent Application No. 08/434,465, filed May 4, 1995, entitled "Nucleic Acid Ligand Complexes," which is incorporated in its entirety herein, describes a method for preparing a therapeutic or diagnostic complex comprised of a nucleic acid ligand and a lipophilic compound or a non-immunogenic, high molecular weight compound.

The methods described herein and the nucleic acid ligands identified by such methods are useful for both therapeutic and diagnostic purposes. Therapeutic uses include the treatment or prevention of diseases or medical conditions in human patients. Diagnostic utilization may include both *in vivo* or *in vitro* diagnostic applications. The

SELEX method generally, and the specific adaptations of the SELEX method taught and claimed herein specifically, are particularly suited for diagnostic applications. SELEX identifies nucleic acid ligands that are able to bind targets with high affinity and with surprising specificity. These characteristics are, of course, the desired properties one skilled in the art would seek in a diagnostic ligand.

The nucleic acid ligands of the present invention may be routinely adapted for diagnostic purposes according to any number of techniques employed by those skilled in the art. Diagnostic agents need only be able to allow the user to identify the presence of a given target at a particular locale or concentration. Simply the ability to form binding pairs with the target may be sufficient to trigger a positive signal for diagnostic purposes. Those skilled in the art would also be able to adapt any nucleic acid ligand by procedures known in the art to incorporate a labeling tag in order to track the presence of such ligand. Such a tag could be used in a number of diagnostic procedures. The nucleic acid ligands to $sPLA_2$, VEGF, and HIV-1 GAG described herein may specifically be used for identification of the $sPLA_2$ and VEGF proteins and the HIV-1 GAG polyprotein.

The SELEX process provides high affinity ligands of a target molecule. This represents a singular achievement that is unprecedented in the field of nucleic acids research. The present invention applies the SELEX procedure to the specific targets of $sPLA_2$, VEGF, and HIV-1 GAG. In the Example section below, the experimental parameters used to isolate and identify the nucleic acid ligands to $sPLA_2$, VEGF, and HIV-1 GAG are described.

In order to produce nucleic acids desirable for use as a pharmaceutical, it is preferred that the nucleic acid ligand (1) binds to the target in a manner capable of achieving the desired effect on the target; (2) be as small as possible to obtain the desired effect; (3) be as stable as possible; and (4) be a specific ligand to the chosen target. In most situations, it is preferred that the nucleic acid ligand have the highest possible affinity to the target.

In co-pending and commonly assigned U.S. Patent Application Serial No. 07/964,624, filed October 21, 1992 ('624), methods are described for obtaining improved nucleic acid ligands after SELEX has been performed. The '624 application, entitled

"Methods of Producing Nucleic Acid Ligands." is specifically incorporated herein by reference.

In the present invention, the SELEX procedure was used to isolate RNAs with specific high affinity for $sPLA_2$ from a degenerate library containing 30 or 50 random positions (30N or 50N). This invention includes the specific RNA ligands to $sPLA_2$ shown in Table 2 (SEQ ID Nos.:10-122). identified by the methods described in Examples 1 and 2.

This invention also includes post-SELEX modified RNA ligands to VEGF having $2'NH_2$ groups on the pyrimidine residues and 2'-O-methyl groups on various purine residues. In addition, nucleotides that contain phosphorothioate backbone linkages were added at the 5' and 3' ends of the ligands in order to reduce or prevent degradation by exonucleases. Internal backbone positions were also identified in which phosphorothioate linkages could be substituted, without the loss of binding affinity, to reduce or prevent endonucleolytic degradation. The post-SELEX modified RNA ligands provided in Table 7 (SEQ ID NOS:126-137) demonstrate an ability to inhibit the activity of exonucleases and endonucleases, without affecting binding affinities.

Further, this invention includes ssDNA ligands to VEGF. The specific ssDNA ligands are shown in Table 11 (SEQ ID NOS:138-209).

In the present invention, two SELEX experiments were also performed in order to identify RNA with specific high affinity for HIV-1 GAG from degenerate libraries containing 50 random positions (50N) (Tables 12 and 14). This invention includes the specific RNA ligands to HIV-1 GAG shown in Tables 13, 15, 18, 19, and 20 (SEQ ID NOS: 229-248, 255-256, 258, 263, 265, 268-285), identified by the methods described in Examples 13 and 14. This invention further includes RNA ligands to HIV-1 GAG which inhibit the function of HIV-1 GAG.

The scope of the ligands covered by this invention extends to all nucleic acid ligands of $sPLA_2$ and HIV-1 GAG, modified and unmodified, identified according to the SELEX procedure. The scope of the invention also extends to modified RNA ligands and all ssDNA ligands to VEGF identified according to the SELEX process. More specifically, this invention includes nucleic acid sequences that are substantially homologous to the ligands shown in Tables 2, 7, 11, 13, 15, 18, 19, and 20. By

substantially homologous it is meant a degree of primary sequence homology in excess of 70%, most preferably in excess of 80%. A review of the sequence homologies of the nucleic acid ligands shown in Table 2 for $sPLA_2$, Tables 7 and 11 for VEGF, and Tables 13, 15, 18, 19, and 20 for HIV-1 GAG shows that sequences with little or no primary sequence homology (e.g., 25% for HIV-1 GAG) may have substantially the same ability to bind $sPLA_2$, VEGF, and GAG, respectively. For these reasons, this invention also includes nucleic acid ligands that have substantially the same structure and ability to bind $sPLA_2$, VEGF, and HIV-1 GAG, as the nucleic acid ligands shown in Tables 2, 7, 11, 13, 15, 18, 19 and 20. Substantially the same ability to bind $sPLA_2$, VEGF, and HIV-1 GAG means that the affinity is within one or two orders of magnitude of the ligands herein. It is well within the skill of those of ordinary skill in the art to determine whether a given sequence--substantially homologous to those specifically described herein--has substantially the same ability to bind $sPLA_2$, VEGF, or HIV-1 GAG.

Inhibition of HIV-1 GAG function by the SELEX -derived ligands includes inhibition of the function of the GAG polyprotein or any of component parts. For example, inhibition of matrix or capsid interactions could inhibit viral assembly. Inhibition of matrix or of vpr incorporation by p6 could lead to viruses that would be unable to integrate and therefore unable to replicate in cells. Inhibition of cyclophilin incorporation by capsid would also be expected to lead to the production of noninfectious virus for reasons mentioned above. Inhibition of nucleocapsid function could inhibit viral RNA packaging which would lead to the formation of noninfectious virus. Inhibition of nucleocapsid could also lead to inhibition of nucleic acid annealing activities. Inhibition of p2 or p1 could lead to inhibition of proteolytic maturation of HIV.

This invention also includes the ligands as described above, wherein certain chemical modifications are made in order to increase the in vivo stability of the ligand or to enhance or mediate the delivery of the ligand. Examples of such modifications include chemical substitutions at the sugar and/ or phosphate and/or base positions of a given nucleic acid sequence. See, e.g., U.S. Patent Application Serial No. 08/117,991, filed September 9, 1993, entitled "High Affinity Nucleic Acid Ligands Containing Modified Nucleotides," specifically incorporated herein by reference. Other

modifications are known to one of ordinary skill in the art. Such modifications may be made post-SELEX (modification of previously identified modified or unmodified ligands) or by incorporation into the SELEX process.

As described above, because of their ability to selectively bind $sPLA_2$, VEGF, and HIV-1 GAG, the nucleic acid ligands to $sPLA_2$, VEGF, and HIV-1 GAG described herein are useful as pharmaceuticals. This invention, therefore, also includes a method for treating inflammation by administration of a nucleic acid ligand capable of binding to $sPLA_2$, a method for treating angiogenesis-mediated diseases by administration of a nucleic acid ligand capable of binding to VEGF, and a method for treating HIV-1 by administration of a nucleic acid ligand capable of binding to HIV-1 GAG.

Therapeutic compositions of the nucleic acid ligands may be administered parenterally by injection, although other effective administration forms, such as intraarticular injection, inhalant mists, orally active formulations, transdermal iontophoresis or suppositories, are also envisioned. One preferred carrier is physiological saline solution, but it is contemplated that other pharmaceutically acceptable carriers may also be used. In one preferred embodiment, it is envisioned that the carrier and the ligand constitute a physiologically-compatible, slow release formulation. The primary solvent in such a carrier may be either aqueous or non-aqueous in nature. In addition, the carrier may contain other pharmacologically-acceptable excipients for modifying or maintaining the pH, osmolarity, viscosity, clarity, color, sterility, stability, rate of dissolution, or odor of the formulation. Similarly, the carrier may contain still other pharmacologically-acceptable excipients for modifying or maintaining the stability, rate of dissolution, release, or absorption of the ligand. Such excipients are those substances usually and customarily employed to formulate dosages for parental administration in either unit dose or multi-dose form.

Once the therapeutic composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, or dehydrated or lyophilized powder. Such formulations may be stored either in a ready to use form or requiring reconstitution immediately prior to administration. The manner of administering formulations

containing nucleic acid ligands for systemic delivery may be via subcutaneous, intramuscular, intravenous, intranasal or vaginal or rectal suppository.

The following Examples are provided to explain and illustrate the present invention and are not intended to be limiting of the invention.

Example 1 describes the various materials and experimental procedures used in Examples 2-8 for the evolution of nucleic acid ligands to $sPLA_2$. Example 2 describes the RNA ligands to $sPLA_2$. Example 3 describes the minimal sequences necessary for high-affinity binding to $sPLA_2$. Example 4 describes the specificity of RNA ligands to human $sPLA_2$. Example 5 describes 2'F modification of 2'OH high-affinity ligands. Example 6 describes the predicted secondary structure of high affinity ligands of $sPLA_2$. Example 7 describes the inhibition of PLA_2-mediated contractions. Example 8 describes inhibition of the $sPLA_2$ enzymatic activity. Example 9 describes the experimental procedures used to generate high-affinity nucleic acid ligands to VEGF. Example 10 describes post-SELEX modifications of VEGF RNA ligands with 2'-O-methyl groups on purines. Additionally, phosphorothioate backbone substitutions were made to reduce or prevent nuclease degradation without effecting binding affinity. Example 11 describes the stability of post-SELEX modified VEGF RNA ligands to *ex vivo* rat tissue degradation. Example 12 describes obtaining ssDNA ligands to VEGF. Example 13 describes the various materials and experimental procedures used in Examples 14 and 15. Example 14 describes the RNA ligands to HIV-1 GAG, affinities the ligands have for HIV-1 GAG, binding of nucleic acid ligands to different sites on the HIV-1 GAG polyprotein, the frequency of matrix and nucleocapsid ligands evolved in two separate SELEX experiements, competition between HIV-1 matrix and HIV-1 nucleocapsid nucleic acid ligands for binding to the HIV-1 GAG polyprotein, binding of nucleic acid ligands to nucleocapsid proteins from diverse HIV-1 strains, minimal sequence requirement for nucleic acid ligands to bind HIV-1 GAG polyprotein, predicted structural basis for recognition of HIV-1 nucleocapsid protein by nucleic acid ligands, predicted structural basis for recognition of HIV-1 matrix proteins by nucleic acid ligands, mutated HIV-1 GAG nucleic acid ligands, and truncated nucleic acid ligands that bind to the HIV-1 GAG protein. Example 15 describes modified 2'-NH_2 pyrimidine RNA ligands to HIV-1 gag.

EXAMPLE 1 - EXPERIMENTAL PROCEDURES FOR 2'-OH AND 2'-NH_2 LIGANDS TO $SPLA_2$

This example provides general procedures followed and incorporated into Examples 2-8 for the evolution of nucleic acid ligands to $sPLA_2$.

A. *Materials*

The human $sPLA_2$, rabbit anti-human $sPLA_2$ polyclonal antibody, and enzymatic and chromogenic substrates used in these experiments were supplied by Eli Lilly & Co. hen egg-white lysozyme (6X crystallized) was provided by Dr. S.C. Gill, Jr and is commercially available. The 2' NH_2 modified CTP and UTP were prepared according to Pieken *et al.* (1991) Science 253:314-317. DNA oligonucleotides were synthesized by Operon. All other reagents and chemicals were purchased from commercial sources.

B. *The SELEX Process: 2'OH RNA*

The SELEX procedure is described in detail in U.S. Patent 5,270,163 and elsewhere. The DNA template used in 2'OH RNA SELEXes contained 30 random nucleotides, flanked by the N1 template 5' and 3' fixed regions (30N1(SEQ ID NO.:1), Table 1). The fixed regions include DNA primer annealing sites for PCR and cDNA synthesis as well as the consensus T7 promoter region to allow *in vitro* transcription. These single-stranded DNA molecules were converted into double-stranded transcribable templates by PCR amplification using the primers indicated in Table 1 (SEQ ID NOS.:2 and 3). PCR conditions were 50 mM KCl, 10 mM Tris-Cl, pH 9, 0.1% Triton X-100, 7.5 mM $MgCl_2$, 1 mM of each dATP, dCTP, dGTP, and dTTP, and contained 25 U/ml of Taq DNA polymerase. Transcription reactions contained 5 mM DNA template, 5 U/µl T7 RNA polymerase, 40 mM Tris-Cl (pH 8.0), 12 mM $MgCl_2$, 5 mM DTT, 1 mM spermidine, 0.002% Triton X-100, 4 % PEG 8000, 2 mM each of 2'OH ATP, 2'OH GTP, 2'OH CTP, 2'OH UTP, and 0.31 mM α-^{32}P 2'OH ATP. SELEX binding reactions are outlined in Table 1. For the binding reactions, RNA molecules were incubated with $sPLA_2$ in TBSC buffer (137 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$, 10 mM Tris-HCl, pH 7.4) for 15 minutes at 37°C. Protein-RNA complexes were separated from unbound RNA by nitrocellulose filter partitioning and bound RNA was isolated from filters by phenol/urea extraction. The RNA was reverse transcribed into cDNA by AMV reverse

transcriptase at 48°C for 60 minutes in 50 mM Tris-Cl pH (8.3), 60 mM NaCl, 6 mM $Mg(OAc)_2$, 10 mM DTT, 100 pmol DNA primer, 0.4 mM each of dNTPs, and 0.4 U/µl AMV RT. PCR amplification of this cDNA resulted in approximately 500 pmol double-stranded DNA, transcripts of which were used to initiate the next round of SELEX.

C. The SELEX Process: 2'NH_2 RNA

For 2'NH_2 SELEXes, DNA templates contained 50 random nucleotides flanked by N7 (50N7 SELEX (SEQ ID NO.:4)) or by N9 (50N9 SELEX (SEQ ID NO.:7)) 3' and 5' fixed regions shown in Table 1. The RNAs transcribed from these templates contained cytidine and uridine in which the 2' OH of the ribose moiety was replaced with a NH_2 group. In these SELEXes, both nitrocellulose partitioning (50N9 SELEX: rounds 6, 7, 10 and 11; 50N7 SELEX rounds 7, 8, 11 and 12) and $sPLA_2$ immobilized on beads via an anti-$sPLA_2$ polyclonal antibody (50N9 SELEX: rounds 1-5, 8 and 9; 50N7 SELEX rounds 1-6, 9 and 10) were used to separate free from bound RNA.

Polyclonal anti-$sPLA_2$ agarose beads were prepared from an ammonium sulfate precipitated, anion exchange purified immunoglobulin fraction of an $sPLA_2$ immunized rabbit. The immunoglobulins were bound to hydrazide activated agarose beads (CARBOLINK Coupling Gel, Pierce, Rockford, IL) according to the manufacturer's instructions. The resulting immunoglobulin density was estimated to be 1.2 mg/ml of gel. Coupling of $sPLA_2$ was accomplished by incubating 50 µl of Ab-gel with 500 µl of 2 µM $sPLA_2$ in TBSC for 2 hours at 37 ° C. The washed gel, which was resuspended in 500 µl of TBSC and stored at 4°C., had a calculated $sPLA_2$ density of 0.2 to 2 pmol/µl of gel, assuming that 1 to 10% of the Ig fraction is anti-$sPLA_2$ Ab and a stoichiometry of 2 molecules of $sPLA_2$ bound per antibody molecule.

For rounds in which immobilized $sPLA_2$ was used to partition unbound RNA from $sPLA_2$/RNA complexes, RNA was incubated with washed $sPLA_2$-gel in a siliconized column for 5 minutes at 37°C, as indicated in Table 1. Unbound RNA was removed by extensive washing with TBSC. Bound RNA was eluted as two fractions; the first fraction was eluted with calcium free buffer, TBS; the second fraction was eluted with free polyclonal anti-$sPLA_2$ Ab in TBS and was processed for use in the following round. The percentage of input RNA eluted by each step is recorded in Table 1. For rounds in which partitioning was accomplished by nitrocellulose filter binding (Table 1),

free $sPLA_2$ and RNA were incubated for 5 minutes at 37°C, filtered through TBSC prewashed nitrocellulose filters and then washed with 3 ml of TBSC.

RNA/$sPLA_2$ complexes absorbed to nitrocellulose filters and fractions eluted from immobilized $sPLA_2$ were heated at 90°C for 5 minutes in 1% SDS, 2% b-mercaptoethanol and extracted with phenol/chloroform. The RNAs were then processed as in the 2' OH SELEXes except that 2' NH2 CTP and 2' NH2 UTP were substituted for CTP and UTP in transcription reactions.

D. *Nitrocellulose Filter Partitioning*

The nitrocellulose filter partitioning method was used as described in SELEX Patent Applications to determine the affinity of RNA ligands for $sPLA_2$ and for other proteins. Filter discs (nitrocellulose/cellulose acetate mixed matrix, 0.45 µm pore size, Millipore) were placed on a vacuum manifold and washed with 5 ml of TBSC buffer under vacuum. Reaction mixtures, containing ^{32}P labeled RNA pools and $sPLA_2$, were incubated in TBSC for 5 minutes at 37°C, filtered, and then immediately washed with 5 ml TBSC. The filters were air-dried and counted in a Beckman liquid scintillation counter without fluor.

The equilibrium dissociation constant, K_d, for an RNA pool or specific ligand that binds monophasically is given by the equation

$$K_d = [P_f][R_f]/[RP]$$

where, [Rf] = free RNA concentration

[Pf] = free Protein concentration

[RP] = concentration of RNA/protein complexes

K_d = dissociation constant

A rearrangement of this equation, in which the fraction of RNA bound at equilibrium is expressed as a function of the total concentration of the reactants, was used to calculate K_ds of monophasic binding curves:

$$q = (P_T + R_T + K_d - ((P_T + R_T + K_d)^2 - 4\,P_T\,R_T)^{1/2})$$

q = fraction of RNA bound

$[P_T]$ = total protein concentration

$[R_T]$ = total RNA concentration

Many ligands and evolved RNA pools yield biphasic binding curves. Biphasic binding can be described as the binding of two affinity species that are not in equilibrium. Biphasic binding data were evaluated with the equation

$$q = 2P_t+R_t+K_{d1}+K_{d2}-[(P_t+X_1R_t+K_{d1})^2-4P_tX_1R_t]1/2$$
$$-[(P_t+X_2R_t+K_{d2})^2-4P_tX_2R_t]^{1/2},$$

where X_1 and X_2 are the mole fractions of affinity species R_1 and R_2 and K_{d1} and K_{d2} are the corresponding dissociation constants. K_ds were determined by least square fitting of the data points using the graphics program Kaleidagraph (Synergy Software, Reading, PA).

E. *Cloning and Sequencing*

During the last round of the SELEX process, PCR of cDNA was performed with primers which contain recognition sites for the restriction endonucleases HindIII and BamHI. Using these restriction sites, the DNA sequences were inserted directionally into the pUC18 vector. These recombinant plasmids were transformed into *E. coli* strain XL-1 Blue (Stratagene, La Jolla, CA). Plasmid DNA was prepared according to the alkaline hydrolysis method (Zhou *et al.* (1990) Biotechniques 8:172-173) and about 100 clones were sequenced with the Sequenase sequencing kit (United States Biochemical Corporation, Cleveland, OH).

F. *Ligand Truncation*

Truncation experiments were carried out to determine the minimal sequence necessary for high affinity binding of the RNA ligands to $sPLA_2$. For 3' boundary determination, RNA ligands were 5' end-labeled with γ-^{32}P-ATP using T4 polynucleotide kinase. 5' boundaries were established with 3' end-labeled ligands using α-^{32}P-pCp and T4 RNA ligase. After partial alkaline hydrolysis, radiolabeled RNA ligands were incubated with $sPLA_2$ at concentrations ranging from 0.04 nM to 28 nM and $sPLA_2$/RNA complexes were separated from unbound RNA by nitrocellulose partitioning. RNA truncates that bound with high affinity were identified on high-resolution denaturing polyacrylamide gels. For each radioactively labeled ligand, two types of ladders were generated to serve as markers: 1) a partial alkaline hydrolysis ladder, and 2) a partial RNase T1 digestion ladder.

EXAMPLE 2 - RNA LIGANDS TO SPLA$_2$

A. *2' OH SELEX*

The primary objectives of the 2' OH SELEXes were 1) to generate high affinity ligands to human sPLA$_2$ and 2) to determine if a non-amplifiable, non-specific competitor could provide selection pressure for high affinity ligands under experimental conditions which, in the absence of competitor, did not detectably enrich for high affinity ligands. Experimental conditions for the Control, Competition, and Standard SELEX procedures are outlined in Table 1. The (Control) SELEX experiment was initiated with 240 nM sPLA$_2$ and a 10-fold molar excess of 2'OH RNA randomized at 30 contiguous positions, 30N1 (SEQ ID NO.:1); these conditions were maintained throughout this SELEX. A second (Competition) SELEX, in which increased stringency was imposed by a high concentration of a non-amplifiable competitor (0.5 mM tRNA), was initiated with an aliquot of the third round Control SELEX RNA. A third (Standard) SELEX, in which stringency was increased by reducing the sPLA$_2$ concentration to 12 nM, while maintaining a 10-fold molar excess of RNA, was started with an aliquot of the fifth round Control SELEX RNA.

The starting pool contained approximately 5 x 10^{14} RNA molecules (500 pmol) and bound sPLA$_2$ with K_d of 240 nM. After 12 rounds, the Standard and Competition SELEX pools bound sPLA$_2$ biphasically; the high affinity species represented about 25% of the molecules in both pools and bound with a K_d of 4 nM. No improvement was observed in the affinity of the Control SELEX RNA. These results validate the non-amplifiable competitor strategy.

The three SELEXes showed no further improvement in affinity in subsequent rounds. Twelfth and fourteenth round cDNAs from the Standard and Competition SELEXes, respectively, were PCR amplified and cloned into pUC18; 51 clones from the Standard and 34 from the Competition SELEX were sequenced. Sequences were aligned manually and are provided in Table 2. The sequences from the 2' OH SELEX from 30N1 RNA are designated by ligand names with numbers only (SEQ ID NOS.:10-59).

B. *2' NH$_2$ SELEX*

In the 2' NH$_2$ SELEXes, two alternate techniques were used to partition unbound RNA from sPLA$_2$/RNA complexes: nitrocellulose filtration and sPLA$_2$ immobilized on

polyclonal anti-$sPLA_2$ beads as outlined in Table 1. The elution of RNA from immobilized $sPLA_2$/RNA complexes was based on the observation that the binding of many ligands to $sPLA_2$ is calcium dependent and on the premise that free polyclonal antibody can compete off bound ligands.

The starting pools for both the 50N7 (SEQ ID NO.:4) and 50N9 (SEQ ID NO.:7) SELEXes contained approximately 5×10^{14} RNA molecules (500 pmol) and bound $sPLA_2$ with K_d's of 71 and 48 nM, respectively. After 11 rounds, the 50N9 SELEX pool bound $sPLA_2$ biphasically; the high affinity species which represented about 67% of the molecules, bound with a K_d of 1.3 nM. Only a marginal improvement in affinity (K_d = 17 nM) was observed for the twelfth round 50N7 SELEX. Eleventh and twelfth round cDNAs from the 50N9 and 50N7 SELEXes, respectively, were PCR amplified with primers containing restriction sites and cloned into pUC18; 40 clones from the 50N9 and 41 from the 50N7 SELEX were sequenced. Sequences were aligned manually and are shown in Table 2. The sequences from the 50N9 RNA SELEX are designated by ligand names including NN (SEQ ID NOS.:60-80) and the sequences from the 50N7 RNA SELEX are designated by ligand names including NS (SEQ ID NOS.:81-96).

C. *RNA Sequences*

Sequences identified by the $sPLA_2$ SELEX procedures described above are shown in Table 2 (SEQ ID NOS.:10-96). In the 2'OH SELEXes, 16 of 51 (Standard SELEX) and 14 of 34 (Competition SELEX) sequenced ligands were unique. A unique sequence is operationally defined as one that differs from all others by three or more nucleotides. In Table 2, the RNA sequences of all 2' OH RNA ligands are shown in standard single letter code (Cornish-Bowden (1985) NAR 13:3021-3030). These clones fall into five sequence families (I-V) and a group of unrelated sequences (Orphans); ligands in all six groups bind with high affinity. In addition, 12 of 38 (50N9) and 12 of 40 (50N7) sequenced ligands were unique (Table 2). All of the 50N9 ligands that bound $sPLA_2$ with high affinity constituted a single sequence family (VI) which is the dominant family (19 of 40) of the 50N9 SELEX. Ligand NS2 which was isolated repeatedly from the 50N7 SELEX and the 2' OH Standard SELEX orphan 60 are related to family VI.

The data in Table 2 define consensus sequences for families I, II, III and VI (SEQ ID NOS.:97-100). The juxtaposition of the conserved sequences of family VI to

the 5' fixed region and the conservation of the AGA by ligand NN2. suggest that these ligands require at least 3 nucleotides of the 5' fixed sequence for high affinity binding. Similarly, the family II alignment suggests that a 5' proximal CUC is necessary for high affinity binding; in some ligands this is a 5' fixed sequence, while in others it is an evolved sequence. Based on the juxtaposition of the conserved sequence of family I and II to the 3' fixed region an analogous logic predicts 3' boundaries within the 3' fixed region for ligands of these families.

The data in Table 2 also show that the distribution of sequence families I-V and orphans is different in the Standard and Competition SELEXes. Family I and V ligands occur frequently (18/51 and 9/51, respectively) in the Standard SELEX but are undetectable (0/34) in the Competition SELEX. A simple explanation for this difference is based on the observation that the high affinity binding of family I and V ligands and orphan 60 (and presumably the other orphans), unlike ligands of families II, III and IV, is calcium dependent. This correlation suggests that the free calcium was titrated by the high concentration of competitor tRNA, thus disallowing enrichment for high affinity, calcium dependent ligands.

D. *Affinities*

The dissociation constants for representative members of families I - VI, orphan and other ligands were determined by nitrocellulose filter binding experiments and are listed in Table 3. Unlike random RNA, all tested ligands from the 2' OH SELEXes bound biphasically. Since the affinity determinations are made under conditions of protein excess, biphasic binding suggests that the ligand exists as two affinity species that are not in equilibrium, presumably these correspond to alternatively folded conformations. In most cases, the affinity of the low affinity species resembles that of random RNA which suggests that one folded conformation binds with high affinity and that all others bind like random RNA. Most high affinity species have dissociation constants from 0.2 to 2 nM which is 1200 to 120-fold improvement over random RNA.

Unlike the 2' OH ligands, the high affinity 2' NH_2 ligands bind monophasically. The lone exception is family VI ligand NN27 (SEQ ID NO:61) which differs from the consensus sequence in having a rigorously conserved G replaced by a U. The affinity of family VI ligands is approximately 1 nM which is about a 50-fold improvement over that

of random 2' NH_2 RNA. The binding characteristics of the 2' OH and 2' NH_2 ligands demonstrate that a priori it is not possible to know if a biphasic population is a collection of biphasic binders or a mixed population of high and low affinity ligands.

EXAMPLE 3 - LIGAND TRUNCATION

To determine the minimal sequence necessary for high-affinity binding to $sPLA_2$, boundary analyses were performed on representative members of Family I (ligand 2 (SEQ ID NO.:11)), Family II (ligands 72 and 72t (SEQ ID NOS.:20 and 33)), Family V (ligands 80 and 87 (SEQ ID NOS.:53 and 54)) , Family VI (ligands NN41, NN11 and NN19 (SEQ ID NOS.:68, 60, and 64, respectively). Data for truncation/boundary determinations are shown in Table 2 (SEQ ID NOS.:101-121). The 3' boundaries of family I and II ligands are located in the 3' fixed region as is that of family V ligands. The position of the 5' boundary of family II ligands is in the 5' fixed region when the conserved CUC is fixed sequence. Similarly, the 5' boundary of family VI ligands is also in the 5' fixed region, while the 5' boundary of ligand 80 is the 5' G of the 5' fixed region. The 3' boundary of family VI ligands coincides with the 3' end of the consensus sequence.

Boundary locations were checked by determining the affinities of truncated ligands that approximate the minimal ligands. Although in general such truncates frequently bind as well as full length ligands, it is not a necessary outcome for at least two reasons. First, the boundary is primarily defined from the difference in affinity of the boundary species and the species that is one nucleotide shorter. Second, boundary experiments examine the affinity of ligands truncated on only one end, while the minimal ligand is truncated from both ends.

Binding affinities for full length and truncated ligands are shown in Table 3. The truncates of ligands 2 and 87 (ligands 2.3 and 87.3, (SEQ ID NOS.:102 and 109) respectively) bind as well as their full length ligands, while truncates of 72, 72t and NN19 (72.c, 72t.2c and NN19.4, (SEQ ID NOS.:105, 106, and 117) exhibit a 5 to 10 fold loss in affinity. Also, the affinity of NN19.4 is restored to nearly full length levels by the addition of as few as six nucleotides to its 3' end (NN19.11, NN19.13, NN19.15 (SEQ ID NOS.:118, 119, and 120). While the low affinity of truncate 87.4 (SEQ ID NO.:110)

confirms that the 5' G of ligands 80 and 87 is essential for high affinity binding, the high affinity of truncate 87.5 (SEQ ID NO:111) shows that the entire 5' fixed sequence is not necessary. The minimal ligand, operationally defined by the traditional boundary experiment, may include sequences that are required for an alternate function (i.e.., proper folding; the ligands must renature in the course of the experiment) or for no function at all. In other words, ligands that are shorter than the minimal ligand may bind with high affinity.

EXAMPLE 4 - SPECIFICITY OF RNA LIGANDS TO HUMAN $sPLA_2$

The affinity of $sPLA_2$ ligands 2, 72, 80, 87, NN19 and NN19.15 (SEQ ID NOS.:11, 20, 53, 54, 64, and 117, respectively) for proteins other than $sPLA_2$ was determined by nitrocellulose partitioning (Tables 4 and 5). Like $sPLA_2$, hen egg white lysozyme, bFGF and elastase are small, highly cationic proteins. Bovine pancreatic PLA_2 is an evolutionarily and structurally related enzyme. The data in Table 4 show that the ligands are highly specific for $sPLA_2$. Specificity is particularly well illustrated by the affinities for bovine pancreatic PLA_2 which is 10^4-fold less than that for human $sPLA_2$. These data show that in general, evolved RNA ligands to $sPLA_2$ bind to other proteins with an affinity similar to that of random RNA.

EXAMPLE 5 - 2'F MODIFICATION OF 2'OH HIGH AFFINITY LIGANDS

It was of interest to determine if 2'OH RNA ligands converted to a nuclease resistant form by the incorporation of 2' modified pyrimidines retained affinity for the target protein. Five 2' OH high affinity ligands to $sPLA_2$ (ligands 11tF, 72F, 73tF, 86tF and 87F (SEQ ID NOS.:37, 21, 32, 50 and 55, respectively) were transcribed with 2' F CTP and 2' UTP in place of CTP and UTP and their binding affinities determined. As shown in Table 3, both qualitative and quantitative changes were observed in the binding characteristics of four ligands; whereas all five bound biphasically as 2' OH RNA, they bound monophasically as 2'F RNA and their affinity was only marginally better (2 to 5 fold) than that of the random 2'F RNA control, regardless of their 2' OH affinity. On the other hand, the binding and inhibition characteristics of ligand 87 were unchanged by 2'F

modification. No degradation of this RNA was observed after incubation in 0.5X serum for 3 hours.

EXAMPLE 6 - SECONDARY STRUCTURE OF HIGH AFFINITY LIGANDS

In favorable instances, comparative analysis of aligned sequences enables deduction of secondary structure and structure-function relationships. Nucleotides that covary according to Watson-Crick base pairing rules are apt to be paired. Sequences that vary in composition and particularly in length are considered to be unimportant for function, while highly conserved sequences are apt to be directly involved in function.

Comparative analysis of family VI sequences yields a hairpin structure with a highly conserved, asymmetrical internal loop. The terminal loop (T-loop) is variable in both length and sequence (except the first and last positions) and is not apt to be directly involved in binding. The I-loop divides the stem in two. The V-stem varies in length (3-5 nucleotide pairs) and sequence. Three of the 5 base pairs are confirmed by Watson-Crick covariation. The C-stem is absolutely conserved. In this structure, the I-loop, C-stem and single stranded tails are critical for binding.

The suggested structure for family III ligands is a two plane G-quartet with a closing double helix, the sequence of which is not conserved. Based on the limited data, the G-quartet loop sequences and a 5' single stranded sequence may be conserved.

Family II contains two very highly conserved sequences: CUUACRG and GCYGAG. Without exception, the R and Y exhibit Watson-Crick covariation which strongly suggests that they are base paired, leading to a core structure consisting of a conserved, bulged stem that has an unpaired G adjacent to the 3' end of the 3' half of the stem. This G may be a loop or a bulged nucleotide.

EXAMPLE 7 - INHIBITION OF PLA_2-MEDIATED CONTRACTIONS

The ligands of the invention are able to selectively block PLA2-mediated contractions of guinea pig lung pleural strips. The procedure described by Snyder *et al.* (Journal of Pharmacology and Experimental Therapeutics (1992) 262:1147-1153) was followed to test the ability of the ligands to inhibit PLA2-induced contraction. Ligand 19.15 (SEQ ID NO: 121) was tested in this assay and showed a dose dependent

inhibition of contraction. The same ligand was tested for its ability to inhibit an arachadonic acid-mediated contraction and did not act differently than the control. This example demonstrates that the ligands of the invention specifically inhibit PLA2-mediated contraction.

EXAMPLE 8 - INHIBITION OF THE SPLA$_2$ ENZYMATIC ACTIVITY

To directly test the ability of the RNA ligands to inhibit sPLA$_2$ enzymatic activity the procedure described by Reynolds *et al.* (Anal. Biochem. (1992) 204:190-197) was followed. Ligands NN11 (SEQ ID NO: 113), NN19 (SEQ ID NO: 114), NN19.11 (SEQ ID NO: 118), NN19.13 (SEQ ID NO: 119), NN19.14 (SEQ ID NO: 120), NN19.15 (SEQ ID NO: 121), 87 (SEQ ID NO: 54), 87F (SEQ ID NO: 55), 87.7 (SEQ ID NO: 112), 72t (SEQ ID NO: 104), 86t (SEQ ID NO: 49) were tested in this assay and showed an inhibitory effect.

The inhibition and affinity data for ligands 87 and 87.7 suggest a ligand with two domains. The minimal ligand, defined by boundary analysis, corresponds to the high affinity binding domain. A second domain, appended to the binding domain, is responsible for inhibition. The inhibition function may not be sequence dependent.

EXAMPLE 9. EXPERIMENTAL PROCEDURES FOR LIGANDS TO VEGF.

This example provides general procedures followed and incorporated into Examples 10-12 for the evolution of nucleic acid ligands to VEGF.

A. *Materials.*

Recombinant human VEGF (165 amino acid form; MW 46,000) was a generous gift from Dr. Napoleone Ferrara (Genentech). All other reagents and chemicals were of the highest purity available and were purchased from commercial sources.

B. *SELEX.*

Essential features of the SELEX protocol have been described in detail in U.S. Patent No. 5,270,163 as well as in previous papers from these laboratories (*See, e.g.*, Schneider *et al.* (1992) J. Mol. Biol. 228:862). Briefly, DNA templates for *in vitro* transcription (that contain a region of thirty random positions flanked by constant

sequence regions) and the corresponding PCR primers were prepared chemically using established solid phase oligonucleotide synthesis protocols.

The random region was generated by utilizing an equimolar mixture of the four unmodified nucleotides during oligonucleotide synthesis. The two constant regions were designed to contain PCR primer annealing sites, primer annealing site for cDNA synthesis, T7 RNA polymerase promoter region and restriction enzyme sites that allow cloning into vectors (Table 6) (SEQ ID NOS:123-125). An initial pool of RNA molecules was prepared by *in vitro* transcription of approximately 200 picomoles (10^{14} molecules) of the double stranded DNA template utilizing T7 RNA polymerase. Transcription mixtures consisting of 100-300 nM template, 5 units/µl T7 RNA polymerase, 40 mM Tris-Cl buffer (pH 8.0) containing 12 mM $MgCl_2$, 5 mM DTT, 1 mM spermidine, 0.002% Triton X-100, 4% PEG were incubated at 37°C for 2-3 hours. These conditions typically resulted in transcriptional amplification of 10 to 100-fold. Selections for high affinity RNA ligands were done by incubating bFGF with RNA for 10-20 minutes at 37°C in 50 ml of phosphate buffered saline (PBS = 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 137 mM NaCl, 2.7 mM KCl, pH 7.4) and then separating the protein-RNA complexes from the unbound species by nitrocellulose filter partitioning (Tuerk, C. and Gold, L. (1990) Science 249:505-510). The selected RNA (which typically amounted to 5-10% of the total input RNA) was then extracted from the filters and reverse transcribed into cDNA by avian myeloblastoma virus reverse transcriptase (AMV RT). Reverse transcriptions were done at 48°C (60 min) in 50 mM Tris buffer (pH 8.3), 60 mM NaCl, 6 mM Mg(OAc)2, 10 mM DTT and 1 unit/µl AMV RT. Amplification of the cDNA by PCR under standard conditions yielded a sufficient amount of double-stranded DNA for the next round of *in vitro* transcription.

C. *Nitrocellulose Filter Binding Assays.*

Oligonucleotides bound to proteins can be effectively separated from the unbound species by filtration through nitrocellulose membrane filters (Yarus, M. and Berg, P. (1970) Anal. Biochem. 35:450-465; Lowary, P.T. and Uhlenbeck, O.C. (1987) Nucleic Acids Res. 15:10483-10493; Tuerk, C. and Gold, L. (1990) supra). Nitrocellulose filters (0.2 µm pore size, Schleicher and Schuell, Keene, NH) were secured on a filter manifold and washed with 4-10 ml of buffer. Following incubations

of ^{32}P labeled RNA with serial dilutions of the protein for 10 min at 37°C in buffer (PBS) containing 0.01% human serum albumin (HSA), the solutions were applied to the filters under gentle vacuum in 45 ml aliquots and washed with 5 ml of PBS. The filters were than dried under an infrared lamp and counted in a scintillation counter.

D. *Equilibrium Dissociation Constants.*

In the simplest case, equilibrium binding of RNA (R) to VEGF (P) can be described by eq. 1,

$$R.P \underset{Kd}{\rightleftharpoons} R + P \quad (1)$$

where Kd =([R][P]/[R.P]) is the equilibrium dissociation constant. Using the mass-balance equations, the fraction of bound RNA at equilibrium (q) can be expressed in terms of measurable quantities (eq. 2),

$$q=(f/2Rt)\{Pt+Rt+Kd-[(Pt+Rt+Kd)^2-4PtRt]^{1/2}\} \quad (2)$$

where Pt and Rt are total protein and total RNA concentrations and f reflects the efficiency of retention of the protein-RNA complexes on nitrocellulose filters. The average value of f for VEGF in our assays was 0.7.

Most RNA ligands exhibited biphasic binding to VEGF. For those ligands, binding of RNA to VEGF is described by a model where total RNA is assumed to be partitioned between two non-interconverting components (R1 and R2) that bind to VEGF with different affinities (eqs 3 and 4).

$$R1.P \underset{Kd1}{\rightleftharpoons} R1 + P \quad (3)$$

$$R2.P \underset{Kd2}{\rightleftharpoons} R2 + P \quad (4)$$

In this case, the fraction of total bound RNA (q) is given by eq. 5.

$$q=(f/2Rt)\{2Pt+Rt+Kd1+Kd2-[(Pt+\chi 1Rt+Kd1)^2-4Pt\chi 1Rt]^{1/2}-[(Pt+\chi 2Rt+Kd2)^2-4Pt\chi 2Rt]^{1/2}\} \quad (5)$$

where $\chi 1$ and $\chi 2(=1-c1)$ are the mole fractions of R1 and R2 and Kd1 and Kd2 are the corresponding dissociation constants.

Internally-labeled RNA ligands used for binding studies were prepared by *in vitro* transcription using T7 RNA polymerase (Milligan *et al.* (1987) Nucl. Acids Res. 15:8783) and were purified on denaturing polyacrylamide gels to ensure size homogeneity. All RNA ligands were diluted to about 1 nM in PBS, denatured at 90°C for 2 minutes, and then cooled on ice prior to incubation with the protein. This denaturation/renaturation cycle performed at high dilution is necessary to ensure that the RNA is essentially free from dimers and other higher order aggregates. Concentrations of the stock solutions of VEGF, from which other dilutions were made, were determined from the absorbance reading at 280 nm using the calculated value for ϵ_{280} of 46,600 $M^{-1}cm^{-1}$ for the VEGF dimer (Gill *et al.* (1989) Anal. Biochem. 182:319). Data sets that define the binding curves were fit to either eq. 2 or eq. 5 by the non-linear least squares method using the software package Kaleidagraph (Synergy Software, Reading, PA).

E. *Information Boundary Determinations.*

High-affinity VEGF ligands were radiolabeled at the 5'-end with γ-^{32}P-ATP (New England Biolabs, Beverly, MA) and T4 polynucleotide kinase (New England Biolabs, Beverly, MA) for the 3'-boundary determinations, or at the 3'-end with α-^{32}pCp and T4 RNA ligase (New England Biolabs) for the 5'-boundary determination. Radiolabeled RNA ligands were subjected to partial alkaline hydrolysis and then selectively bound in solution to VEGF at 5, 0.5, or 0.125 nM before being passed through nitrocellulose filters. Retained oligonucleotides were resolved on 8% denaturing polyacrylamide gels. In each experiment, the smallest radiolabeled oligonucleotide bound by VEFG at the lowest protein concentration defines the information boundary. Partial digests of the 5'- or the 3'-labeled RNA ligands with RNAse T_1 (Boehringer Mannheim Biochemicals, Indianapolis, IN) were used to mark the positions of labeled oligonucleotides ending with a guanosine.

F. *Cloning and Sequencing.*

Individual members of the enriched pool were cloned into pUC18 vector and sequenced as described (Schneider, D. *et al.* (1992) J. Mol. Biol. 228:862-869).

G. *Receptor Binding.*

VEGF was radioiodinated by the Iodegen method (Jakeman *et al.* (1992) J. Clin. Invest. 89:244) to a specific activity of 2.4 x 10^4 cpm/ng. Human umbilical vein endothelial cells (HUVECs) were plated in 24-well plates at a density of 1-2 x 10^5 cells/well and grown to confluence in EGM (Clonetics, San Diego, CA) media (24-48 hrs). At confluence, the cells were washed 3 times with PBS and incubated for 2 hrs at 4°C in α-MEM serum-free media containing ^{125}I-labeled VEGF with or without unlabeled competitor (VEGF, EGF, or RNA). For experiments done with RNA, 0.2 units of placental RNase inhibitor (Promega, Madison, WI) were included in the media. It was determined that the RNA ligands were not degraded during the course of the experiment. At the end of the 2 hr incubation period, the supernatant was removed and the wells washed 2 times with PBS. HUVECs were then lysed with 1% triton X-100/1 M NaOH and the amount of cell-associated ^{125}I-VEGF determined by gamma counting.

EXAMPLE 10 - POST SELEX MODIFICATIONS OF VEGF RNA LIGANDS.

VEGF RNA ligands are described in WO 95/07364 (PCT/US94/10306), which is incorporated herein by reference in its entirety. In an attempt to further stabilize the nucleic acid ligands of the ligands described in WO 95/07364, certain post-SELEX modifications were done. The ligand NX107 (SEQ ID NO:126) was chosen as a model for post-SELEX modification. NX107 is a truncated version of Ligand 24A, which is described in WO 95/07364. All of the pyrimidines in NX107 have an NH_2 group substituted at the 2'-position of the ribose. This example describes substitution of O-Methyl groups at the 2'-position of the ribose of certain of the purines of NX107. Additionally, phosphorothioate nucleotides were added at the 5' and 3' ends of the ligands and in at least one instance, at an internal position. The various substitutions to the ligand were designed to inhibit the activity of exonucleases and endonucleases, but not affect binding affinity.

To this end, certain ligands were synthesized and tested for binding affinity. The sequences and the results of the binding studies are provided in Table 7. The binding studies were performed using the protocols described in Example 9.

EXAMPLE 11 - STABILITY OF POST-SELEX MODIFIED VEGF LIGANDS TO EX VIVO RAT TISSUE DEGRADATION.

In order to be able to quickly assess the effects of ligand modifications on stability to tissue nucleases, the following assay was developed. Brain, kidney, liver and spleen tissues were removed from a freshly sacrificed rat, washed in saline to remove blood, and sliced into approximately 10 mm^3 pieces. Each piece was put into an Eppendorf tube with 50 μl PBS and quick frozen on dry ice. Tissues from the same rat were used for all the experiments described here. The ligand to be tested was 5'end-labeled with ^{32}P, added to the thawed tissue slice in 80μl PBS, and incubated at 37°C. Aliquots were withdrawn at 3, 10, 30, and 60 minutes, added to an equal volume of formamide dyes on ice, and quick-frozen on dry ice. The samples were run on a 20% denaturing acrylamide gel along with equal counts of the unincubated ligand, and a partial alkaline hydrolysate of the ligand (or a related ligand) for sequence markers. The gels were dried and exposed to X-ray film and a phosphorimager plate (for quantitation of degradation).

The VEGF ligands used in this study are shown in Table 7. Each ligand has the same core 24-mer sequence derived from a truncated 2'NH_2-pyrimidine SELEXed ligand (NX-107)(SEQ ID NO:126). NX-178 (SEQ ID NO:128) is the same 2'amino pyrimidine ligand with phosphorothioate backbone linked thymidine caps at the 5'- and 3'- ends of the ligand. NX-190 (SEQ ID NO:129) is an all DNA version of the same sequence with the above-described caps, and NX-191 (SEQ ID NO:130) is an all 2'OMe version. NX-213 (SEQ ID NO:131) is the capped amino ligand with all the purines 2'OMe substituted except four. NX-215 (SEQ ID NO:133) is the same as NX-213 with an internal phosphorothioate linkage between A7 and U8.

Tables 8 and 9 provide the results obtained by this assay on rat brain and kidney tissues as indicated by the percent of full length material found at the various time points. For this analysis, a ligand is still considered functionally intact with cuts in the

phosphorothioate caps. The other tissues assayed had similar results. The post-SELEX modifications were successful in protecting the ligand from various endo- and exonucleases.

EXAMPLE 12 - SSDNA LIGANDS TO VEGF.

This example demonstrates the ability to obtain ssDNA ligands to vascular endothelial growth factor (VEGF).

Most of the materials and methods are the same as those described in Example 9. Two libraries of synthetic DNA oligonucleotides containing 40 random nucleotides flanked by invariant primer annealing sites were amplified by the Polymerase Chain Reaction (PCR) using oligonucleotide primers as shown in Table 10 (SEQ ID NOS:216-221). The protocols for the SELEX procedure are as described by Jellinek *et al.* (PNAS (1993) 90:11227-11231), in the SELEX Patent Applications and in Example 9. VEGF protein binding assays, receptor binding assays, and information boundary determinations are also described in Example 9.

The ssDNA ligands identified are shown in Table 11 (SEQ ID NOS:138-199). Only the sequence of the evolved region is provided in Table 11, however, each of the clones also includes the fixed regions of either SEQ ID NO:216 or SEQ ID NO:219. Clones named with numbers only include the fixed regions of SEQ ID NO:216 and clones named with b and number included the fixed regions of SEQ ID NO:219. Truncations (information boundary determinations) were performed on a number of ligands, which is also provided in Table 11 (SEQ ID NOS:200-209). Four sequence families were obtained from the alignment of the primary sequences of these ligands and a consensus sequence generated for each family (SEQ ID NOS:210-215). Orphan sequences were also identified. Select ligands were tested in the VEGF protein binding assay with results being shown in Table 11. The starting DNA random pool had a binding affinity Kd of approximately 200 nM. In the VEGF receptor binding assay, the truncated clone 33t (SEQ ID NO:203) had a Ki of 3 nM.

EXAMPLE 13. EXPERIMENTAL PROCEDURES FOR LIGANDS TO HIV-1 GAG

This example provides general procedures followed and incorporated into Examples 14-15 for the evolution of nucleic acid ligands to HIV-1 GAG.

A. *Materials.*

The HIV-1 p55 (gag), p7 (nucleocapsid), and p15 (p7 nucleocapsid-p6) proteins from HIV-1 strain LAI (generous gifts from Drs. Tristram Parslow and Jared Clever (Dept. of Pathology and the Dept. of Microbiology and Immunology, University of California, San Francisco, CA 94143-0506)) were expressed in *E. coli* as fusions to glutathione S-transferase (GST) and purified as described (Clever *et al.* (1995) J.Virol. 69:2101-2109). They are referred to in the Examples as GST-gag, GST-p7 and GST-p15, respectively. The GST protein itself was also purified using the same procedures. The HIV-1 p24 capsid and HIV-1 $p7_{MN}$ proteins were obtained from the NIH AIDS Research and Reference Reagent Program. The HIV-1 p17 matrix protein was obtained from the MRC AIDS Reagent Project. All oligonucleotides used were synthesized by Operon, Inc. (Alameda, CA). Restriction enzymes were purchased from New England Biolabs (Beverly, MA). All other reagents, chemicals and plasmids were from commercial sources.

B. *SELEX ligand generation.*

SELEX ligands that bind to the HIV-1 gag polyprotein were derived in two SELEX experiments ("A" and "B") essentially as described in U.S. Patent No. 5,270.163 (see also Tuerk and Gold (1990) Science 249:505-510; Tuerk *et al.* (1992) Proc. Natl. Acad. Sci. USA 89:6988-6992; Tuerk and MacDougal-Waugh (1993) Gene 137:33-39; Tuerk *et al.* (1994) in The Polymerase Chain Reaction, eds. Ferre *et al.* (Birkhauser, Springer-Verlag, New York; Jenson *et al.* (1994) J. Mol. Biol. 235:237-247) with the following modifications. SEQ ID NO.222 was used as the starting template and SEQ ID NOS: 223 and 224 were used for the PCR primers for SELEX "A" and SEQ ID NO. 249 was used as the starting template and SEQ ID NOS. 250 and 251 were used as the PCR primers for SELEX "B." SELEX experiment "A" used a starting RNA pool of 8.4×10^{14} molecules with 50 random bases (Table 12; SEQ ID NO: 225), a binding buffer that consisted of 50 mM Tris, pH 7.5, 200 mM KOAc, 5 mM $MgCl_2$, 1 mM dithiothreitol, and avian myeloblastosis virus (Life Sciences, Inc., St. Petersburg, FL) as the reverse

transcriptase. SELEX experiment "B" used a starting RNA pool of 7 X 10^{14} molecules with 50 random bases (Table 14; SEQ ID NO: 252), a gag binding buffer that consisted of 50 mM Tris, pH 7.4, 140 mM KCl, 5 mM NaCl, 5 mM $MgCl_2$, 1mM dithiothreitol (gag binding buffer) and murine leukemia virus reverse transcriptase (Superscript, Gibco, Inc., Gaithersburg, MD) as the reverse transcriptase. SELEX experiment "B" also utilized increasing concentrations of heparin (molecular weight 5,000, Calbiochem, Inc., San Diego, CA) at a 10-200 fold excess over the RNA pool in each round of SELEX to compete with nonspecifically binding RNAs in the pool for binding to gag. Both the "A" and the "B" SELEX used Taq polymerase (Perkin-Elmer Cetus, Inc., Norwalk, CT) to amplify cDNA products. Binding of RNA pools was for 5 minutes in SELEX "A" and for 10-15 minutes in SELEX "B." T7 RNA polymerase and 2'-hydroxyl nucleosides were used to transcribe the amplified cDNAs. Nitrocellulose partitioning was used for both SELEX experiments except for round 5 in SELEX "B." Column SELEX was used for round 5 in SELEX "B" as described infra.

C. *Subcloning of ligands.*

The ligand pool from the appropriate round was amplified using PCR primers (Table 12; SEQ ID NOS: 226 and 227) for SELEX "A," and Table 14, (SEQ ID NOS: 253 and 254) for SELEX "B" that have restriction enzyme recognition sites on the 5' and 3' ends. The PCR products were digested with the appropriate restriction enzyme (*HindIII* and *BamHI* for SELEX "A" and BamHI and EcoRI for SELEX "B") and then subcloned into pUC9 (BRL, Inc., Gaithersburg, MD) that was also digested with the appropriate restriction enzymes. The ligation was transformed into *E.coli* strain DHα.

D. *Sequence analysis of clones.*

Plasmids were prepared from the transformants using either the boiling method or the alkaline lysis method using art known techniques (see Molecular Cloning: A Laboratory Manual (Eds. Sambrook *et al.* (1989). The plasmids were denatured with 0.5 M NaOH, desalted on a 0.5 ml G-50 Sephadex spin column, and then used for sequencing. Sequences were determined using the Sequenase version 2.0 enzyme (U. S. Biochemical, Inc., Cleveland, OH) according to manufacturer's instructions. A DNA primer labeled with phosphorous-32 at the 5'-end and corresponding to the T7 promoter (Table 12; SEQ ID NO: 228) was used as the sequencing primer.

E. *Binding affinities of ribonucleic acid ligands for target HIV-1 proteins.*

The binding affinities of ribonucleic acid ligands for the various proteins (i.e., GST-gag, GST-p7, matrix, capsid, GST, GST-p15) were measured by filtration essentially as described (Tuerk and Gold (1990) Science 249:505-510; Jellinek *et al.* (1994) Biochemistry 33:10450-10456). Briefly, radiolabeled RNA was transcribed from a PCR-generated template using T7 RNA and polymerase and α-^{32}P-ATP (DuPont NEN Research Products, Boston, MA). The RNA (~200 fmol; ~10,000 cpm) was mixed with the protein at concentrations ranging from 10^{-12} to 10^{-6} M at 37 °C. for 5-10 minutes in a 30 μl reaction. When the protein used was GST-p7, GST-p15 or GST-gag, a 10,000 fold molar excess of yeast tRNA was added to the RNA prior to mixing with the protein. The buffer used for binding is as described above. The reactions were vacuum-filtered through nitrocellulose filters (HAWP, Millipore, Inc. Bedford, MA). A control with no protein added was also included for each RNA to correct for the amount of the RNA that binds to the nitrocellulose filter instead of protein. The amount of labeled RNA specifically retained on the filter by protein was determined, and the apparent K_d of the protein for the RNA was obtained by plotting the amount of RNA specifically bound to protein vs. the concentration of the protein using Kaleidograph computer software (Synergy, Inc., Reading, PA).

As a positive control for binding to gag, an RNA referred to herein as Ψ-456 was used (see Example 13). Ψ-456 contains the HIV-1 RNA packaging signal (Ψ) which binds specifically to the HIV-1 gag and nucleocapsid proteins during the course of viral assembly.

The Ψ-456 RNA was made by transcription of Xho I-digested pBsP (Luban and Goff (1991) J.Virol. 65: 3203-3212) using T7 RNA polymerase. Ψ-456 RNA consists of 441 bases from the HIV-1_{HXB2R} RNA (containing nucleotide positions 712-1152 of HIV-1_{HXB2R} RNA; (Myers *et al.* eds. (1993) Human retroviruses and AIDS 1993: A compilation and analysis of nucleic acid and amino acid sequences, Los Alamos Natl. Lab., Los Alamos, NM) plus 15 bases of vector-derived sequence at the 5' end. Ψ-70 (containing nucleotide positions 712-765 of HIV-1_{HXB2R} RNA) was transcribed from a double-stranded DNA template generated by a polymerase chain reaction using pBsP as a template. Ψ-93 (containing nucleotide positions 712-788 of HIV-1_{HXB2R} RNA) was

transcribed from Nde I-digested pBsP. Ψ-136 (containing nucleotide positions 712-831 of HIV-1_{HXB2R} RNA) was transcribed from Cla I-digested pBsP.

F. *Competition binding reactions.*

Nucleic acid ligands (SW8.27, SEQ ID NO:236; SW8.4, SEQ ID NO:231; SW10.12, SEQ ID NO:242; SW8.24, SEQ ID NO:234) were transcribed with T7 RNA polymerase as described above using α-^{32}P-ATP. Each radiolabeled ligand was mixed with various amounts of unlabeled competitor RNAs (SW8.27, SEQ ID NO:236; SW8.4, SEQ ID NO:231; Ψ-456, or the starting RNA pool for SELEX "A", SEQ ID NO:225) ranging in concentration from 10^{-12} to 10^{-6} M. The mixed RNAs were then added to the appropriate HIV-1 protein at a concentration of protein equal to the affinity (apparent K_d) of the protein for the radiolabeled RNA ligand. The reaction was incubated in gag binding buffer and then filtered as described above.

G. *Ligand boundary determinations.*

The boundaries of nucleic acid ligands were determined essentially as described by Jellinek *et al.* (1994) Biochemistry 33:10450-10456 with the following modifications. The concentration of the appropriate HIV-1 protein that was used was equal to the apparent K_d. The HIV-1 p17 matrix protein was used to determine the boundaries of matrix nucleic acid ligands and the GST HIV-1 p15 (nucleocapsid-p6) protein was used to determine the boundaries of nucleocapsid nucleic acid ligands. In some binding reactions, especially with high affinity ligands, a 10,000 fold molar excess of heparin or yeast tRNA over the ligand was added to suppress nonspecific binding to short RNA fragments. For the RNase T_1 digestion of ligands, yeast tRNA was added at a concentration of 0.3 mM and then incubated for 5 minutes at 37 °C.

H. *Truncated and mutated nucleic acid ligands.*

Truncated nucleic acid ligands were generated by one of two methods. In one method, an oligonucleotide consisting of the antisense strand of the T7 ϕ10 promoter plus the antisense sequence of the desired truncated ligand was synthesized. This was hybridized to an oligonucleotide with the sequence corresponding to the sense strand of the T7 ϕ10 promoter. Such complexes can be used as a template for transcription by T7 RNA polymerase to generate truncated nucleic acid ligands (Milligan *et al.* (1987) Nuc. Acids Res. 15:8783-98; Milligan and Uhlenbeck (1989) Methods Enzymol. 180:51-62).

In another method. two oligonucleotides were synthesized. The sequence of one oligonucleotide contained the sense sequence of the T7 RNA polymerase ϕ10 promoter and part of the 5' end of the truncated ligand. The sequence of the second oligonucleotide encoded the antisense sequence of the 3' end of the truncated ligand. The oligonucleotides were designed so that they overlapped. These oligonucleotides were subjected to 3 rounds of the polymerase chain reaction in order to generate a double-stranded T7 RNA polymerase transcription template.

Mutated nucleic acid ligands were generated in a similar manner by incorporating the desired base changes into the oligonucleotides used to generate the transcription templates.

EXAMPLE 14. RNA LIGANDS TO HIV-1 GAG

A. *In vitro evolution of RNAs that bind to gag.*

A GST-gag fusion protein was used in both SELEX experiments. GST alone has a low affinity (K_d >10 μM) for random RNA, whereas GST-gag has a higher affinity (K_d =0.1-1 μM) for random RNA. Therefore, as a result of the properties of the SELEX method, the majority of the evolved ligands bind to gag and not GST.

SELEX "A" was carried out for a total of 11 rounds. The sequence of the RNA pool was determined at rounds 5, 6, and 7 and found to be nonrandom. The K_d of the round 10 pool was determined and found to be 1.5 nM. In the same experiment, the K_d of Ψ-456, the HIV-1 gag-binding RNA element, was 1.1 nM. On the basis of the K_d and the nonrandomness of the sequences, the ligand pools from rounds 8 and 10 were cloned into pUC9 for sequence analysis of individual ligands.

Sequences were obtained from 34 clones, 19 from round 8 and 15 from round 10. Twelve of the sequences were identical and are represented by ligand SW8.4 (SEQ ID NO: 231). One ligand, represented by ligand SW10.22 (SEQ ID NO: 243), was almost identical to the ligand SW8.4 family. Two other sequences were identical to ligand SW8.6 (SEQ ID NO: 232). Another ligand, SW8.27 (SEQ ID NO: 236) differs from SW8.6 by two bases. Ligands SW8.24 (SEQ ID NO: 234) and SW10.39 (SEQ ID NO: 247) differ by one base. In summary, from 34 clones, 23 (SEQ ID NOS: 229-248) unique sequences were obtained.

The 23 unique ligands were screened for binding to the GST gag protein at 0.0nM, 0.1 nM and 10.0 nM gag, concentrations that bracket the average Kd of the round 10 pool. Round 0 and Ψ-456 RNA were added as negative and positive controls, respectively, for binding to gag. All of the ligands were also filtered in the absence of GST-gag (0.0nM) to detect nitrocellulose binding sequences. The binding was done in the presence of a 10,000 fold molar excess of yeast tRNA. Three ligands were found to be nitrocellulose binders. The data for the 20 ligands that bound to GST-gag are summarized in Table 16. Several ligands bound well to GST-gag, especially the most frequent ligand SW 8.4 (and the related ligand SW 10.22), SW 8.1, SW 8.27, and SW 10.28.

It has been reported previously that ligands which bind to nitrocellulose filters are rich in guanine. The gag ligands from SELEX "A" that bound to nitrocellulose had 18 or more guanines in the randomized region, whereas the gag ligands that bound to gag had 14 or less guanines in the randomized region. The sequences of 20 clones from rounds 8 and 10 of SELEX "A" that bound to the gag protein are shown in Table 13.

SELEX "B" was carried out for a total of 9 rounds. After 4 rounds of SELEX, the amount of RNA that was binding to nitrocellulose filters had risen. Therefore, we employed an alternate partitioning strategy for round 5. Round 5 was performed using "column SELEX." 0 or 10 nmoles GST-gag protein was bound to 50 ml glutathione Sepharose 4B (Pharmacia) on ice for 15 minutes. 150 nmoles of round 5 RNA pool was added and incubated at 37° C. for 15 minutes. The beads were pelleted in a microfuge at 1000 rpm for 2 minutes. The aqueous supernatant was saved. The pelleted beads were washed in 1 ml of ice cold gag binding buffer and then the beads were immediately repelleted. The beads were washed in such a manner for a total of 6 times. All of the washes and the beads were Cherenkov-counted. At this point, the beads incubated with GST-gag bound 180 fmoles RNA, while the beads with no GST-gag prebound had bound 3.3 fmoles RNA. The beads were then washed with 2-250 µl aliquots of gag binding buffer containing 5 mM reduced glutathione to elute GST-gag RNA complexes. The eluted GST-gag RNA complexes were phenol extracted, ethanol precipitated, and the recovered RNA was reverse-transcribed, amplified by PCR, and transcribed (as described in Tuerk and Gold (1990) Science 249:505-510) in preparation for the round 6

selection. The enrichment of ligands in this round was calculated to be as much as 830 fold. substantially more than that for other rounds in which a standard nitrocellulose partitioning was used (average of about 100 fold enrichment).

The sequence of the RNA pools in SELEX B were determined at rounds 0, 2, 4, 6, and 8. The sequences of rounds 0, 2, and 4 were random. The sequences of the RNA pool from rounds 6 and 8 were nonrandom. The high degree of enrichment that may have been achieved in round 5 using column SELEX may explain the sudden shift in the sequence of the RNA pools from random in round 4 to nonrandom in round 6.

The K_d of the round 0, 4, and 8 pools was determined and found to be ~1 μM, 8.5 nM, and 1.1 nM, respectively. In the same experiment, the K_d of Ψ-456, the HIV-1 gag-binding RNA element, was 5.2 nM.

The ability of Ψ-456, round 4, and round 8 RNA pools from SELEX "B" to compete with Ψ-456 for binding to gag was examined. The concentration of ^{32}P-labeled Ψ-456, round 4, and round 8 RNA pools was approximately 150 pM, the concentration of GST-gag was fixed at approximately 1 nM, and the concentration of unlabeled Ψ-456 was varied. The amount of unlabeled Ψ-456 required to compete off 50% of the ^{32}P-labeled Ψ-456, round 4, or round 8 RNA pools was a 20, 74 or 630 fold excess, respectively. Therefore, SELEX ligands can compete for binding to gag more effectively than Ψ-456. Since SELEX-derived gag ligands have a lower K_d for gag than Ψ-456 and since they compete more effectively for binding than does Ψ-456, in a gene therapy setting SELEX-derived gag ligands may not need to be expressed to a level comparable to the HIV-1 RNA in order to effectively compete with Ψ for binding to gag.

On the basis of the K_d and the nonrandomness of the sequence pools after round 6, the ligand pool from rounds 6 and 8 of SELEX "B" were subcloned into pUC9 for sequence analysis of individual ligands.

Nine unique sequences from round 6 and 13 unique sequences from round 8 of SELEX "B" were found (Tables 15 and 18). Seven clones were identical to ligand ML8.7 (SEQ ID NO: 255) and two more, represented by ligand ML8.14 (SEQ ID NO: 258) were similar to ligand ML8.7. The ligands from SELEX "B" were also screened for binding to GST-gag or nitrocellulose filters as described above. The results are shown in Table 18.

Both SELEX "A" and "B" had a ligand that was more frequent in occurrence than the others. In SELEX "A" the most frequent ligand was represented by SW8.4 (SEQ ID NO: 231). This clone or closely related ligands were present in 13 of 27 (48%) gag-binding sequences obtained. In SELEX "B", the most frequent ligand was ML8.7 (SEQ ID NO: 255). This clone or closely related ligands were represented in 9 of 21 (42%) gag-binding sequences obtained. Thus in each SELEX experiment about 40-50% of the clones were a single dominant ligand. The most frequent clones from each of the two SELEX experiments were not highly similar to each other (17/50 evolved bases when optimally aligned). This was expected since different templates and conditions were used in the two SELEXs.

B. *Affinity of ribonucleic acid ligands that bind to the HIV-1 gag protein.*

The nucleic acid ligands (SEQ ID NOS:268-276) that bound specifically and to a significant extent to GST-gag and not to nitrocellulose filters were analyzed further over a wider range of GST-gag concentrations to obtain an apparent binding affinity (K_d). These results are shown in Table 17. Most of the ligands bound to gag with a K_d near 1-10 nM. Ψ-456 typically bound to GST-gag with a K_d near 1 nM. Therefore, the gag nucleic acid ligands can bind to gag at least as well as the native HIV-1 Ψ element.

Some of the affinity of Ψ-456 for GST-gag could be due to a length affect. We attempted to use shorter forms of Ψ-456 that were closer in length to that of the GST-gag ligands (97-98 bases). Forms of Ψ-456 were generated that were 70, 93, or 136 bases long (see Example 13), but these did not bind as well as Ψ-456 to GST-gag (K_d= 500, 500, and 10 nM respectively) or to the same extent. Therefore, Ψ-456 was used in the various binding and competition binding experiments reported here.

C. *Binding of nucleic acid ligands to different sites on the HIV-1 gag polyprotein.*

Regions of GST-gag to which the RNA ligands bind were determined. Twenty three GST-gag ligands were screened for binding to GST, HIV-1 capsid, HIV-1 nucleocapsid (GST-p7 or GST-p15), and HIV-1 matrix proteins. The binding specificity of the nucleic acid ligands that bind to the gag protein fell mainly into two classes, those that bind matrix and those that bind to nucleocapsid (GST-p7) (Table 18).

Binding to nucleocapsid was expected since it is a known RNA binding protein. Binding to matrix was considered to be possible since the avian leukosis retrovirus

matrix protein was reported to bind RNA (Leis *et al.* (1980) J.Virol. 35:722-731) and also because the HIV-1 matrix protein is basic (pI=9.9). In the NMR structure of matrix (Massiah *et al.* (1994) J.Mol.Biol. 244:198-223); Matthews *et al.* (1994) Nature 370:666-668) there is a basic patch of amino acids near the amino terminus. These amino acids are involved in the nuclear import of the integration machinery. It is possible that the matrix nucleic acid ligands bind to this region.

One nucleic acid ligand, ML8.11 (SEQ ID NO. 256), bound to GST. In addition one ligand, ML6.17 (SEQ ID NO. 276), bound to GST-gag with high affinity, but did not bind to the p17 matrix, p24 capsid, GST-p7 (nucleocapsid), GST-p15 (nucleocapsid-p6), or GST proteins. It is possible ML6.17 binds to more than one protein component within GST-gag. This would explain its inability to bind to the separate protein components of GST-gag.

D. *Frequency of matrix and nucleocapsid ligands evolved in two separate SELEX experiments.*

Nucleocapsid ligands were evolved more frequently in SELEX "A" and matrix ligands were evolved more frequently in SELEX "B" (see Table 18). In SELEX "A" nucleocapsid ligands represented 74% (20 of 27) and matrix ligands represented 26% (7 of 27) of the total HIV-1 gag ligands. In SELEX "B" matrix ligands represented 56% (10 of 18) and nucleocapsid ligands represented 33% (6 of 18) of the total. This can be explained because heparin was used as a competitor of nonspecific or lower affinity protein/RNA interactions in SELEX "B". As is the case for RNA, heparin also has a higher affinity for the HIV-1 nucleocapsid protein than for the HIV-1 matrix protein. Having heparin present in SELEX "B" may have blocked an RNA binding site on the HIV-1 nucleocapsid protein to allow more frequent evolution of RNAs that bind to an RNA binding site on the HIV-1 matrix protein.

Heparin and possibly other polyanionic molecules (e.g., tRNA) can be used to manipulate the proportion of matrix and nucleocapsid ligands that are evolved using the SELEX method. In this manner one could increase the efficiency at which either matrix or nucleocapsid ligands are isolated. Furthermore, if one desired to use the Ψ element as a competitor in a SELEX experiment, for example, to attempt to improve the affinity or specificity of nucleocapsid ligands, it would be necessary to use the nucleocapsid protein

rather than gag. If gag were used in such an experiment, Ψ would bind to the nucleocapsid nucleic acid binding site and one would risk obtaining only matrix ligands.

D. *Competition between HIV-1 matrix and HIV-1 nucleocapsid nucleic acid ligands for binding to the HIV-1 gag polyprotein.*

Since ligands were evolved that bind to two separate sites on the HIV-1 gag protein (matrix and nucleocapsid), it was investigated whether ligands that bound to the separate sites would compete with each other for binding. Competition binding reactions were done between matrix ligands, nucleocapsid ligands, and Ψ-456. Homologous ligands interfered with each other for binding to gag. That is, a matrix ligand (SW8.27, SEQ ID NO:236) interfered with the binding of another matrix ligand (SW10.12, SEQ ID NO:242) and a nucleocapsid ligand (SW8.4, SEQ ID NO:231) interfered with the binding of another nucleocapsid ligand (SW8.24, SEQ ID NO:234). This indicates there may be only one binding site for RNA on matrix or nucleocapsid, but that there are at least two RNA binding sites on the gag protein. All ligands competed off the binding of the starting pool of RNA (SEQ ID NO:225) at lower concentrations.

On the other hand, heterologous ligands did not interfere with each other for binding to gag. A matrix ligand (SW 8.27, SEQ ID NO:236) did not interfere with the binding of a nucleocapsid ligand (SW 8.4, SEQ ID NO:231) or Ψ-456. Conversely a nucleocapsid ligand (SW 8.4, SEQ ID NO:231) did not interfere with the binding of a matrix ligand (SW 8.27, SEQ ID NO:236). Therefore at least some matrix and nucleocapsid ligands may be able to bind to gag simultaneously. This could provide an opportunity for simultaneous, combination delivery of matrix and nucleocapsid ligands to the same cell in order to effect a treatment for HIV-1 infection. That matrix ligands and nucleocapsid ligands bind to different sites on gag might be expected since matrix and nucleocapsid are at opposite ends of an elongated protein (Nermut *et al.* (1994) Virology 198:288-296).

It is notable that a HIV-1 nucleocapsid nucleic acid ligand does interfere with the binding of Ψ-456 to the gag polyprotein. This might be expected since Ψ is known to bind specifically to the HIV-1 nucleocapsid protein. Since HIV-1 nucleocapsid nucleic acid ligands interfere with the binding of Ψ, nucleocapsid-specific nucleic acid ligands derived by the SELEX method can function as "decoys" (i.e., compete with Ψ for

binding to the nucleocapsid protein) and therefore can be useful in the treatment of HIV-1 infection.

E. *Binding of nucleic acid ligands to nucleocapsid proteins from diverse HIV-1 strains.*

The ability of nucleic acid ligands to bind to nucleocapsid protein from different HIV-1 strains was investigated by binding nucleic acid ligand ML6.6 (SEQ ID NO.:271) to a HIV-1 nucleocapsid protein from either a macrophage-tropic MN or a T-cell tropic LAI strain of HIV-1. These two proteins differ by 3 amino acids out of a total of 55. The ligand bound best to the LAI form of nucleocapsid and had about a 10 fold lower affinity for the MN form of the HIV-1 nucleocapsid protein. This indicates there may be some preference for a nucleic acid ligand to bind to the nucleocapsid protein it was evolved to bind to, but that they can also bind to other nucleocapsid proteins. Because nucleocapsid is a small, highly conserved protein, it is thought to be less likely to mutate so as to become resistant to inhibitors that bind to it (Rice *et al.* (1995) Science 270:1194-1197). Thus, drugs that bind to the HIV-1 nucleocapsid protein may be particularly useful for treating HIV infection.

F. *Minimal sequence required for nucleic acid ligands to bind to the HIV-1 gag polyprotein.*

In order to learn more about what sequences might be in common among matrix or nucleocapsid ligands that might enable them to bind to gag and what the structural basis for binding to gag might be, the minimal sequences were determined that are required for some of the ligands to bind to the HIV-1 gag protein with high affinity. This information was obtained through boundary determination and truncation studies. The results of these experiments are shown in Tables 19 and 20.

Boundary experiments were performed on nine nucleocapsid ligands and three matrix ligands. The lengths of the predicted minimal ligands range from 14 to 59 bases. Seven of the ligands required part of the fixed region sequence to bind to gag, while five required only evolved sequences to bind to gag.

G. *Structural basis for recognition of HIV-1 nucleocapsid protein by nucleic acid ligands.*

The minimal nucleocapsid binding sequences (Table 19) all form relatively stable ($\Delta 6$ = -2 to -20 Kcal/mol) stem-loop structures. In addition, the boundaries of all nine

nucleocapsid ligands examined end at a palindromic sequence that ranges in size from 8-16 bases. Usually such palindromes are at the 3' boundary. The majority of the palindromes were 8-11 bases in length. Some ligands contain more than one palindrome. In such cases, it is the palindrome that is near 8-11 bases long that is near the boundary. Often a UGU-purine sequence is also found near the palindrome, but in some cases this sequence is not absolutely required for high affinity binding to nucleocapsid. Many of the palindromes contain smaller 4-base palindromes at their ends. This suggests the nucleic acid ligands that bind to the HIV-1 nucleocapsid protein may interact through palindromes to form dimers. Of course, the stem-loop structures could also anneal to form dimers. The exact role of the palindrome in binding of ligands to nucleocapsid is not known, but their presence in all nucleocapsid ligands indicates they are important. The presence of palindromes fits well with the documented complementary strand annealing properties of nucleocapsid (You and McHenry (1994) J.Biol.Chem. 269:31491-31495; Lapadat-Tapolsky *et al.* (1995) Nuc.Acids Res. 23:2434-2441). However, it is not known if the nucleic acid ligands reported here form dimers in solution in the absence of nucleocapsid or whether nucleocapsid enhances the rate of their formation.

The binding properties of several truncates of SW8.4 (SEQ ID NO.:231) support the theory that palindromes are involved in the binding of the nucleocapsid ligands to gag and nucleocapsid. Ligands that do not contain palindromes (e.g., matrix ligands) do not bind to nucleocapsid. Also ligands with mutations in the palindrome do not bind to nucleocapsid (see below).

The occluded site size of nucleocapsid can vary from about 7-15 bases, depending on the buffer conditions and the protein/nucleotide ratio (Khan and Giedroc (1994) J.Biol.Chem. 269:22538-22546; You and McHenry (1994) J.Biol.Chem. 269:31491-31495). This size is strikingly similar to the size of the palindromes observed in the sequences evolved here. Six of the nine nucleocapsid ligands have palindromes that are 8-11 bases long. This lends further support that the palindromes observed in the nucleocapsid ligands are of significance with respect to binding to the HIV-1 gag polyprotein. Thus the sequences of nucleic acid ligands that bind to gag appear to be

most representative of the initial interactions between nucleocapsid and the Ψ region *in vivo* (annealing of complementary strands between the loop region of kissing loops).

Alternative nucleocapsid binding motifs that were considered include guanine quartets (Sundquist and Heaphy (1993) Proc.Natl.Acad.Sci.USA 90:3393-3397) and kissing loops (Paillart *et al.* (1994) J.Biol.Chem. 269:27486-27493); Skripkin *et al.* (1994) Proc.Natl.Acad.Sci.USA 91:4945-4949); Laughrea and Jetté (1994) Biochemistry 33:13464-13474; Muriaux *et al.* (1995) J. Biol. Chem. 270:8209-8216; Laughrea and Jetté (1996) Biochemistry 35:1589-1598), which have been proposed to be involved in the binding of Ψ to gag. There has been some controversy about what the important sequence elements are for recognition of Ψ by gag and for RNA packaging.

Different elements may be required for distinct dimer formation, gag binding, and RNA packaging steps. The involvement of guanine quartets was ruled out as a mechanism for the binding of HIV-1 nucleocapsid nucleic acid ligands because there were no consistent sequence patterns found in all HIV-1 nucleocapsid nucleic acid ligands that would suggest guanine quartets could form. In addition, the binding of one nucleocapsid ligand (SW8.4; SEQ ID NO. 231) to GST-gag was not sensitive to salt as would be expected if guanine quartets were involved. SW8.4 bound to gag as well in lithium or sodium as in the potassium used in the SELEX combinatorial chemistry procedure. Potassium has been reported to dramatically stabilize guanine quartets.

It was possible that the binding of ligand SW8.4 involved a kissing loop since a sequence within SW8.4 (positions 29-55) can form a kissing loop-like structure that is structurally similar to the proposed kissing loop in Ψ. However, the involvement of kissing loops in the binding of SW8.4 to the HIV-1 nucleocapsid protein was ruled out because the boundary of SW8.4 does not include the entire kissing loop and the truncated SW8.4t30-53 ligand, which does not include all the kissing loop, still binds to nucleocapsid.

H. *Structural basis for recognition of HIV-1 matrix protein by nucleic acid ligands.*

A common sequence motif could be discerned among the three minimal HIV-1 matrix nucleic acid ligands examined. The consensus sequence motif is:

PuXPyAGX$_6$PuGXPuXCAXPuXPuPuXGXUGXA (SEQ ID NO:289)

The entire sequence of each minimal matrix ligand is predicted to form a stable stem/loop structure. The CAXPu motif is found in the predicted loop region of each. With the exception of matrix ligand ML8.7 (SEQ ID NO. 255), other matrix ligands (including SW8.27 (SEQ ID NO. 236) which was not amenable to boundary determination) do not contain palindromes. The palindrome within the boundary of ML8.7 does not fit the size and position observed for those found in nucleocapsid ligands. Therefore, the sequence and structural motifs required by matrix-specific nucleic acid ligands for binding to gag are different from those required by nucleocapsid specific ligands.

1. *Mutated HIV-1 gag nucleic acid ligands.*

Mutations in two ligands were constructed in order to study the role of putative sequence elements in their ability to bind to the HIV-1 gag protein.

As discussed above, the matrix ligands are predicted to form a stem/loop structure with a CAXPu motif near the loop. Although the role of sequences within the matrix motif in GAG binding have not been studied extensively, deletion of a GC dinucleotide overlapping the CAXPu motif (GCAAG) in ligand ML6.8 (SEQ ID NO. 272) eliminated binding to matrix (see Table 21). This supports the notion that conserved nucleotides in the consensus matrix ligand sequence are important and that sequences in the loop may be particularly important.

Two versions of ligand SW8.4 (See Table 21) were constructed and tested for binding to GST-p7 (see Table 21). Ligand SW8.4G47C is a full length version of SW8.4 that has a single point mutation at position 47. This mutation is in the palindrome proposed to be involved in recognition of the nucleocapsid protein. This mutation eliminated the ability of SW8.4 to bind to nucleocapsid. Ligand SW8.4 GUCCAU (45-50) GCGCGC was constructed to determine if replacement of the proposed loop (GUCCAU) in the proposed kissing loop of SW8.4 by the sequence (GCGCGC) of the proposed kissing loop of Ψ would still allow SW8.4 to bind to GST-gag. This mutation is a triple mutant relative to the wild type sequence. In addition, since the loop is also the same as the palindrome proposed to bind nucleocapsid, this mutant also introduces a triple mutation into the palindrome. This triple mutant also eliminated the ability of SW8.4 to bind to nucleocapsid.

J. *Truncated nucleic acid ligands that bind to the HIV-1 gag protein.*

Nine truncated nucleic acid ligands were generated based on the boundary data shown in Table 19 in order to validate the results of the individual 5' and 3' boundary experiments. These were tested for binding to the appropriate HIV-1 protein. The results are shown in Table 20. Six truncated nucleic acid ligands were identified. Five of these bind to the HIV-1 nucleocapsid protein and one binds to the HIV-1 matrix protein.

All five truncated nucleocapsid ligands which correspond to the predicted minimal boundaries bind to GST-p15 nucleocapsid. The smallest truncate tested that bound to nucleocapsid was 29 bases long, but in theory a 14 base long truncate of ligand SW8.25 (SEQ ID NO. 235) may be able to bind to nucleocapsid.

Two truncates of ligand SW8.4 were used to distinguish between the kissing loop model and a palindrome model for ligand binding. Ligand $SW8.4_{NC}t41$-55 (SEQ ID NO. 283) contains the top stem/loop of the predicted kissing loop, but does not bind to nucleocapsid. Ligand $SW8.4_{NC}t30$-53 (SEQ ID NO. 284) is missing the entire 3' sequence of the stem of the predicted kissing loop and thus would not be able to form a kissing loop. Nevertheless, it does bind to nucleocapsid, possibly because it contains the entire palindromic region and includes the predicted minimal ligand based on boundary experiments.

One truncated matrix ligand which corresponds to the predicted minimal boundaries binds to the HIV-1 matrix protein. This truncate tested that bound to matrix was 50 bases long.

EXAMPLE 15 - MODIFIED 2'-NH2 PYRIMIDINE RNA LIGANDS TO HIV-1 GAG.

In order to generate ligands with improved stability *in vivo*, an experiment is carried out with randomized RNA containing NH_2 functionalities at the 2'-position of each pyrimidine. A library of 7-9 X 10^{14} molecules is generated that contains 50 nucleotides of contiguous random sequence flanked by defined sequences. Defined nucleotide sequences in the flanking regions of the template can serve as primer annealing sites for PCR and with one of the primers providing the T7 promoter sequence (a restriction site can be added for cloning). The random and fixed nucleotides of the

initial candidate mixture are comprised of 2'-NH_2 pyrimidine bases. The round of selection and amplification are carried out as described supra in Examples 13-14 using art-known techniques.

TABLE 1
SELEX CONDITIONS

Random RNA

SEQ ID NO:	Name	Sequence
1	30N1 RNA	5' gggagcucagaauaaacgcucaa-30N-uucgacaugaggcccggauccggc 3'
2	N1 5' Primer	5' ccgaagcttaatacgactctatagggagctcagaataaacgctcaa 3'
3	N1 3' Primer	5' gccggatccgggcctcatgtcgaa 3'
4	50N7 RNA*	5' gggaggacgaugcgg-50N-cagacgacucgcccga 3'
5	N7 5' Primer	5' taatacgactcactatagggaggacgatgcgg 3'
6	N7 3' Primer	5' tcgggcgagtcgtcctg 3'
7	50N9 RNA*	5' gggaaaagcgaaucauacacaaga-50N-gcuccgccagagaccaaccgagaa 3'
8	N9 5' Primer	5' taatacgactcactatagggaaaagcgaatcatacacaaga 3'
9	N9 3' Primer	5' ttctcggttggtctctggcggagc 3'

*All C and U have 2'NH_2 substituted for 2'OH for ribose

2'OH SELEX CONDITIONS

30N1 SELEX

SELEX	Round	[$sPLA_2$]	[RNA]	[tRNA]
Control	1-14	240 nM	2.4 μM	-
Standard	1-5	240 nM	2.4 μM	-
	6-12	12 nM	120 nM	-
Competition	1-3	240 nM	2.4 μM	-
	4-14	240 nM	2.4 μM	0.5 mM

TABLE 1 (PAGE 2)

2'NH_2 SELEX CONDITIONS

50N9 & 50N7 SELEX

IMMOBILIZED $sPLA_2$ PARTITIONING

Round	sPLA2 (pmol)	RNA (pmol)	Gel Vol	Total Vol	TBSC Eluted RNA	Ab Eluted RNA
1	0.2 - 2	468	1 ul	0.5 ml	2.6%	1.9%
2	0.2 - 2	100	1 ul	1.0 ml	3.0%	2.6%
3	0.4 - 4	345	2 ul	1.0 ml	0.1%	0.03%
4	0.4 - 4	540	2 ul	1.0 m	0.05%	0.03%
5	2 - 20	730	10 ul	1.0 ml	0.07%	0.03%
8	2 - 20	90	10 ul	1.0 ml	1.0%	0.2%
9	2 - 20	102	10 ul	0.5 ml	2.4%	0.3%

NITROCELLULOSE PARTITIONING

R und	[$sPLA_2$]	[RNA]	Vol	Net RNA bound
6	25 nM	373 nM	1.5 ml	2.1%
7	10 nM	100 nM	1.5 ml	2.1%
10	5 nM	50 nM	1.5 ml	3.8%
11	5 nM	50 nM	1.5 ml	7.0%

TABLE 2*

Family I

- **Ligands**

10 71 gggagcucagaauaaacgcucaa------UCUCAU-GCUCGUCGCACGGCGUAACCUAUuucgacaugaggcccggauccggc
11 2(6) gggagcucagaauaaacgcucaa------UCUCAUUGCUCGUCGCACGGCGUAACCUAUuucgacaugaggcccggauccggc
12 3 gggagcucagaauaaacgcucaa-----AGCUCAUCGUC--UCGCAAGGCGUAUCCUAUuucgacaugaggcccggauccggc
13 77 gggagcucagaauaaacgcucaaACCUCGCCU-AU-GU---UCGCCGCGGCGUAUCCUAu-ucgacaugaggcccggauccggc
14 93(3) gggagcucagaauaaacgcucaa---CAGCCA-AU-GUGUCCCGUACGGCGUAUCCUAu-ucgacaugaggcccggauccggc
15 95 gggagcucagaauaaacgcucaa---CGCUGGAU-ACCA-UCGCACGGCGUAUCCUGCuucgacaugaggcccggauccggc
16 85 gggagcucagaauaaacgcucaa--AUUGCAUCAU-GUA--CCGCAAGACGUAUUCUAu-ucgacaugaggcccggauccggc
17 18 gggagcucagaauaaacgcucaa--AUCGCAUCAU-GUA--CCGCAAGACGUAUUCUAu-ucgacaugaggcccggauccggc
18 54 gggagcucagaauaaacgcucaa--AUUGCAUCAU-GUA--CCGUAAGACGUAUUCUAu-ucgacaugaggcccggauccggc
19 14 gggagcucagaauaaacgcucaa--AUUGCAUCAU-GUA--CCGCAAGACGUAUCCUAuuucgacaugaggcccggauccggc
97 Consensus Sequence YMUCAU-GUH--YCGYAMGRCGUAUYCUAU
Boundary Species
101 2 CUCAUUGCUCGUCGCACGGCGUAACCUAUuucgacauga
Truncates and Derivatives
102 2.3 GG------UCUCAUUGCUCGUCGCACGGCGUAACCUAUuucgacaugag

Family II

- **Ligands**

20 72(6) gggagcucagaauaaacgcucaaGACCUCUG----CUUACAG------CCCG-----GCUGAGACAC----------uucgacaugaggcc cggauccggc
21 72F gggagcucagaauaaacgcucaaGACCUCUG----CUUACAG------CCCG-----GCUGAGACAC----------uucgacaugaggcc cggauccggc
22 96(2) gggagcucagaauaaacgcucaaGACCUCUG----CUUACAG------UUCG-----GCUGAGACAC----------uucgacaugaggc ccggauccggc
23 13t gggagcucagaauaaacgcucaaUGCCUCUG----CUUACGG------GUAAU----GCCGAGACAC----------uucgacaugaggcc cggauccggc
24 94 gggagcucagaauaaacgcucaaAGUCCUCUC----CUUACGG------UUCG-----CCCGAGAUAA----------uucgacaugaggc ccggauccggc
25 15 gggagcucagaauaaacgcucaaGACCUCUG----CUUACAG------CCCG-----GCUGAGACAU----------uucgacaugaggcc cggauccggc
26 79 gggagcucagaauaaacgcucaaGACCUCUG----CUUACAG------CUCG-----GCUGAGACAC----------uucgacaugaggcc cggauccggc
27 88 gggagcucagaauaaacgcucaaGACCUCUG----CUUACAG------UCCG-----GCUGAGACAC----------uu cgacaugaggcccggauccggc
28 81 gggagcucagaauaaacgcucaaGACCUCUG----CUUACAG------CCCG-----GCUGAGACGC----------uucg acaugaggcccggauccggc
29 68t gggagcucagaauaaacgcucaaGGCCUCUG----CUUACGG------CUAAU----GCCGAGACGC----------uucg acaugaggcccggauccggc
30 75 gggagcucagaauaaacgcucaaGANCUCUG----CUUACAG------CCCG-----GCUGGGACAC----------uucg acaugaggcccggauccggc
31 73t gggagcucagaauaaacgcucaa----CUUACAG------UUCG-----GCUGAGAGAAGACGCAUACuucgacaugagg cccggauccggc

TABLE 2 (PAGE 2)

32	73tF	gggagcucagaauaaacgcucaa----CUUACAG------UUCG-----GCUGAGAGAAGACGCAUACuucgacaugagg cccggauccggc
33	72t(2)	gggagcucagaauaaacgcucaa----CUUACAG------UUCG-----GCUGAGACGAAGAUCGACCuucgacaugagg cccggauccggc
34	71t(3)	gggagcucagaauaaacgcucaa----CUUACAG--GAGAUUCCAUCUCGCUGAGACGC----------uucgacaugagg cccggauccggc
35	1t	gggagcucagaauaaacgcucaa----CUUACGGCAGCGAUUGCUG---GCCGAGAAACC----------uucgacaugagg cccggauccggc
36	11t(2)	gggagcucagaauaaacgcucaa----CUUACGG------GUAAA----GCCGAGAAAAUGUAUUGC-uucgacaugagg cccggauccggc
37	11tF	gggagcucagaauaaacgcucaa----CUUACGG------GUAAA----GCCGAGAAAAUGUAUUGC-uucgacaugagg cccggauccggc
38	36t(9)	gggagcucagaauaaacgcucaaUUGUCUUACAG------GUAAA----GCUGAGGAAUCGUU-----uucgacaugagg cccggauccggc
39	4(6)	gggagcucagaauaaacgcucaa-UGUCUUACGG------GUAAA----GCCGAGAAAGUUUCC----uucgacaugag gcccggauccggc
40	35t(3)	gggagcucagaauaaacgcucaaGGCUGGG-UCUUUUACAG------GUAAA----GCUGAGAA-------------uucga caugaggcccggauccggc
41	89	gggagcucagaauaaacgcucaa-AGUCUUACGG------GUAAA----GCCGAGAAAGUUUCC----uucgacaugagg cccggauccggc
42	84t	gggagcucagaauaaacgcucaaUCAUGUC-AUUACGG------GUAAA----GCCGAGUUUUC----------uucgacau gaggcccggauccggc43
43	79t	ggagcucagaauaaacgcucaaAUCAUGUC-AUUACGG------GUAAA----GCCGAGUUUUC----------uucgaca ugaggcccggauccggc
98	Consensus Sequence	CUCWR----CUUACRG------BYMV-----GCYGAGA
	Boundary Species	
103	72	aGACCUCUG----CUUACAG------CCCG-----GCUGAGACAC----------uucgacaugagg
104	72t	uaaacgcucaa----CUUACAG------UUCG-----GCUGAGACGAAGAUCGACCuucgaca
	Truncates and Derivatives	
105	72.c	GGGaaGACCUCUG----CUUACAG------CCCG-----GCUGAGACAC----------uucgacaugaggcc
106	72t.2c	GGGauaaacgcucaa----CUUACAG------UUCG-----GCUGAGACGAAGAUCGACCuucgacau
	Family III	
	• Ligands	
44	34t(2)	gggagcucagaauaaacgcucaaCACGAGGGUGGGUGGCCGAGCGCUUGuucgacaugaggcccggauccggc
45	9t	gggagcucagaauaaacgcucaaCACGGGGGUGGGUGGCCGAGCGCUUGuucgacaugaggcccggauccggc
46	6t	gggagcucagaauaaacgcucaaUGCCUCAUGCCAAUGUGGGAGGGUGGGUGGuucgacaugaggcccggauccggc
47	76t	gggagcucagaauaaacgcucaaCGCCUCAUGCCAACGCGGGAGGGUGGGUGGuucgacaugaggcccggauccggc
48	83t	gggagcucagaauaaacgcucaaCGCCUCAUGCCAACGUGGGAGCGUGGGUGGuucgacaugaggcccggauccggc
49	86t	gggagcucagaauaaacgcucaaCGCCUCAUGCCAAUGCGGGAGGGUGGGUGGuucgacaugaggcccggauccggc
50	86tF	gggagcucagaauaaacgcucaaCGCCUCAUGCCAAUGCGGGAGGGUGGGUGGuucgacaugaggcccggauccggc
99	Consensus Sequence	MAYGNGGGWGGGUGGGUGG

TABLE 2 (PAGE 3)

Family IV		
•	**Ligands**	
51	2t	gggagcucagaauaaacgcucaaUCCGGGAGCUGAAAAA-CAUGCCGUUAGCCGuucgacaugaggcccggauccggc
52	70t	gggagcucagaauaaacgcucaaUCCGCGAGCUGAAAAA-CAUGCCGUUAGCCAuucgacaugaggcccggauccggc
Family V		
•	**Ligands**	
53	80(7)	gggagcucagaauaaacgcucaaGCUCUGGGAUGAUGCCCAGUGUCCAGCAUCuucgacaugaggcccggauccggc
54	87(2)	gggagcucagaauaaacgcucaaGCUCUGGGAUGAUGCCCACUGUCCAGCAUCuucgacaugaggcccggauccggc
55	87F	gggagcucagaauaaacgcucaaGCUCUGGGAUGAUGCCCACUGUCCAGCAUCuucgacaugaggcccggauccggc
	Boundary Species	
107	80	gggagcucagaauaaacgcucaaGCUCUGGGAUGAUGCCCAGUGUCCAGCAUCuucgac
108	87.3	gggagcucagaauaaacgcucaaGCUCUGGGAUGAUGCCCACUGUCCAGCAUCuucgac
	Truncates and Derivatives	
109	87.3	gggagcucagaauaaacgcucaaGCUCUGGGAUGAUGCCCACUGUCCAGCAUCuucgacaug
110	87.4	ggagcucagaauaaacgcucaaGCUCUGGGAUGAUGCCCACUGUCCAGCAUCuucgacaug
111	87.5	gggagcu----auaaacgc---aGCUCUGGGAUGAUGCCCACUGUCCAGCAUCuucgacaug
112	87.7	gggagcucagaauaaacgcucaaGCUCUGGGAUGAUGCCCACUGUCCAGCAUCuucgacauguGUAGCUAAACAGCUUUAGGA
Orphan Sequences		
•	**Ligands**	
56	1	gggagcucagaauaaacgcucaaGCCGAACCGAAUGGAGGUGGAGGGAUUGCGuucgacaugaggcccggauccggc
57	84	gggagcucagaauaaacgcucaaGACCACGUCCGAACGAACACCGCCACGCAuucgacaugaggcccggauccggc
58	90	gggagcucagaauaaacgcucaaCCAACGACACUCACGCAUUGCCCACGAACGuucgacaugaggcccggauccggc
59	60	gggagcucagaauaaacgcucaaACAACGGCCCACACGGGAGAUCCGAGAAAAGuucgacaugaggcccggauccggc
Family VI		
•	**Ligands**	
60	NN11(5)	gggaaaagcgaaucauacacaagaCCGGCCGGGGAAA---CCCGAGGUCCGAGGUAACGCAUGGCGCCUCACCGAGUCgcuccgccag agaccaaccgagaa
61	NN27	gggaaaagcgaaucauacacaagaCCGGCCGGGGAAA---CCCGAGGUCCGAUGUAACGCAUGGCGCCUCACCGAGUCgcuccgccagag accaaccgagaa
62	NN16	gggaaaagcgaaucauacacaagaCCGGCCGGGGAAA---CCCGAGAUCCGAGGUAACGCAUGGCGCCUCACCGAGUCgcuccgccagag accaaccgagaa
63	NN10	gggaaaagcgaaucauacacaagaCCGGCCGGCGCCAUA-GCCGAGAUCCGAGGUUGUACGAUGACAACUCAGUgcuccgccagagacca accgagaa
64	NN19	gggaaaagcgaaucauacacaagaCCGGCCGGCGCCAUA-GCCGAGAUCCGAGGUGUUGAACGAUAACUCGGUgcuccgccagagaccaa ccgagaa
65	NN2(4)	gggaaaagcgaaucauacacaagaGACCGGCCAGCCAAGGC-GCUGAGAUCCGAGGUUUCAGAACCCAUCGGGUUGgcuccgccagaga ccaaccgagaa

TABLE 2 (PAGE 4)

```
66   NN11d     gggaaaagcgaaucauacacaagaCCGGCCCGGUAUGUAGCCGGAGAUCCGAGACUUGCUGAACGAGGUGCCACGgcuccgccagagacc aaccgagaa
67   NN29      gggaaaagcgaaucauacacaagaCCGGCCCGGUGUGUAGCCGGAGAUCCGAGACUUGCUGAACGAGGUGCCACGgcuccgccagagacc aaccgagaa
68   NN41      gggaaaagcgaaucauacacaagaCCGGCCCGGUGUGCAGCCGGAGAUCCGAGACUUGCUGAACAAGGUGCCACGgcuccgccagagacc aaccgagaa
69   NN5(2)    gggaaaagcgaaucauacacaagaCCGGCCCCGCCAAUCAAGGGAGAUCCGAGGAAUUGGAAUGUUUGUGAGUGAgcuccgccagagacc aaccgagaa
100   Consensus Sequence            ragaCCGGCCNGSNNNNNNNNSCNGAGAUCCGAGG

      Boundary Species
113  NN11                    aagaCCGGCCGGGGAAA---CCCGAGGUCCGAGGUAACGCA
114  NN19                    aagaCCGGCCGGCGCCAUA-GCCGAGAUCCGAGGUGUUG
115  NN2                     gaGACCGGCCAGCCAAGGC-GCUGAGAUCCGAGGUUUCA
116  NN41                    aagaCCGGCCCGGUGUGCAGCCGGAGAUCCGAGACUUGCUG

      Truncates and Derivatives
117  NN19.4  gggaaaag-----------aagaCCGGCCGGCGCCAUA-GCCGAGAUCCGAGGUGUUGA
118  NN19.11 gggaaaag-----------aagaCCGGCCGGCGCCAUA-GCCGAGAUCCGAGGUGUUGAACGAU--------------------agac caaccgagaa
119  NN19.13 gggaaaag-----------aagaCCGGCCGGCGCCAUA-GCCGAGAUCCGAGGUGUUG-------------------------agac caaccgagaa
120  NN19.14 gggaaaag-----------aagaCCGGCCGGCGCCAUA-GCCGAGAUCCGAGGUGUUGAACGAU---------------------- ---ccgagaa
121  NN19.15 gggaaaag-----------aagaCCGGCCGGCGCCAUA-GCCGAGAUCCGAGGUGUUG---------------------------- ---ccgagaa
122  NS2(9)             gggaggacgaugcggUAGUAACGGA----UACUGAUCcGAGGUUAUAGUCACUAUAUCAUCCGCUGCGCcagacgacucgcccga

Other Sequences
 •
70   NN3    gggaaaagcgaaucauacacaagaGUGUGCGGUGCAUGCGUGGUGAAAGGGGGGUGGGGAAGAAAAACCGGCCCgcuccgccagagacca accgagaa
71   NN4(9)   gggaaaagcgaaucauacacaagaGUGUGCGGUGCAUGCGUGGUGAAAGGUGGGUUGUGGAGGAAGACCGUGCCgcuccgccagagacca accgagaa
72   NN1      gggaaaagcgaaucauacacaagaCGGCGAGCAUGCGGCGAGUGGAGGGGGACUGAUGGAGGGCGAGACCGUGUgcuccgccagagacca accgagaa
73   NN6      gggaaaagcgaaucauacacaagaCGGCGAGCAGGCGGCGAGUGGAGGAGGACUGAUGGAGGGCGAGACCGCAGgcuccgccagagacca accgagaa
74   NN7      gggaaaagcgaaucauacacaagaCGGCGAGCAAGCGGCGAGUGGAGGAGGACUGAUGGAGGGCGAGACCGCAAgcuccgccagagacca accgagaa
75   NN22      gggaaaagcgaaucauacacaagaCGGCGAGCAUGCGGCGAGUGGAGGAGGACUGAUGGAGGGCGAGACCGUGUgcuccgccagagacca accgagaa
76   NN45     gggaaaagcgaaucauacacaagaCGGCGAGCAGGCGGCGAGUGGAGGAGGACUGAUGGAGGGCGAGACCGCGUgcuccgccagagacca accgagaa
77   NN40(2)  gggaaaagcgaaucauacacaagaCCCCUUGAGCUCGUGACGCAGGAGGAGGGCCGAGGAGGAAAGUCGUCACAgcuccgccagagac caaccgagaa
78   NN18     gggaaaagcgaaucauacacaagaGGAUGGCGGCAAGGCGCGAAAGGGAGGAUCGAGGAGGAAUCGCGUCAGGAgcuccgccagagacca accgagaa
79   NN30     gggaaaagcgaaucauacacaagaGCCAGCGAGUGUCGACAGUGUGGGUGGAAGUGACGGGAGGAUUGGAGACgcuccgccagagaccaa ccgagaa
80   NN39     gggaaaagcgaaucauacacaagaCUGGUUGUGCGGACCCAGUGAGUGGGAGGACGUGAGGGUGGCAGCUGGgcuccgccagagaccaac cgagaa
```

60

TABLE 2 (PAGE 5)

81	NS20(17)	gggaggacgaugcggAGGGUGGAUCGUGGAGGAAAAGCAUCGUGUGUAACCGAACCGAUCGUGGYcagacgacucgcccg a
82	NS39(3)	gggaggacgaugcggGUAGGGAUGAAGUGCGAUGUGAAUCCGGGUGCUAGCGAUGAUGUGUGCCCcagacgacucgcccg a
83	NS38(2)	gggaggacgaugcggGUAGGGAGACAGACACACACGCGGAAAGUAGAGCCAUCGUAACAUGCCCCcagacgacucgcccg a
84	NS4	gggaggacgaugcggGUAGGGAUAAGCGAGUGUACAGCGAAUACGACUCGGAAUGCUUGGUGCGCcagacgacucgcccg a
85	NS12(2)	gggaggacgaugcggUGAAAGAGAAAGGUUGAGAUGAUUACAAGCGAAUUGGAUAAGUGUCUGGCcagacgacucgcccg a
86	NS27	gggaggacgaugcggUGAAAUGAGAAAUGGAUUGAUGAUGAUUAcaagagAAUUGGAUAAGUGUCUGGUcagacgacucg cccga
87	NS11(2)	gggaggacgaugcggGAGGGAAGGGUGGAACGGAACUCCGAUAAAGCUGUACAAGUACGUGGGGUcagacgacucgcccg a
88	NS1	gggaggacgaugcggAUAAGGAGGAGCAAGCGAGAAAUUGAGAAGUAACAAGAUCGACAUGGCCCcagacgacucgcccg a
89	NS49	gggaggacgaugcggAUAAGGAUAAGAUCGAACGAGAGUGAACAAAGUUAAAUACAGUCUGGGGGcagacgacucgcccg a
90	NS48	gggaggacgaugcggGCUAAGGGAAGACAAUGAGAUAGCAGACAAUCAACUACACCCAUGUGCGUcagacgacucgcccg a
91	NS13	gggaggacgaugcggUGAGCUUAGGAUAGGAGCAACAAGUAGAGUAGAGUGAUAACUAGGGUGGCcagacgacucgcccg a
92	NS10	gggaggacgaugcggUGACAAAUGAGCAAGUAGCGAUAGAUGUGAUGGACAGAGACAGCCGGGGCcagacgacucgcccg a
93	NS14	gggaggacgaugcggAAAUGUGUUAGUGAAUGAUUGAGAGAAGAUAGAUGAUGUUGAAGUCUGGCcagacgacucg cccga
94	NS6	gggaggacgaugcggAACuAAAAGACAGAGAGAAAACGACAAuACGAAGuACAuAuAACCCuGGCcagacgacucgcccg a
95	NS18	gggaggacgaugcggAAGUAGAUGAUGGAUUGAGAUGUAAGUGUCAGUAUGAAGAGUCUCUGGGCcagacgacucgcccg a
96	NS7	gggaggacgaugcggAGGAAAUGAAGUAGUGAGAGUAUAACAUGAUUAUGAAUACGUGAUGUGGCcagacgacucgcccg a

*Fixed sequences are represented by lower case lettering; evolved sequence by upper case. "-" indicates spacing for alignment. Ligands recovered from the 2'OH Competition SELEX are denoted by "t"; F indcates 2'OH ligands which contain 2'F CTP and 2'F UTP in place of CTP and UTP, respectively. Sequences that were isolated more than once, are indicated by the parenthetical number, (n), following the ligand isolate number.

- Sequence ID Number

TABLE 3 (Page 1)

A: 2'OH Ligands

Ligand	SEQ ID NO	Kd1	Kd2	Mole fraction
Family I				
71	10	1.3 nM	82 nM	0.60
2	11	1.7 nM	180 nM	0.45
2.3	102	2.6 nM	740 nM	0.55
3	12	1.9 nM	33 nM	0.55
85	16	3.8 nM	92 nM	0.60
93	14	17.2 nM	82 nM	0.50
95	15	4.5 nM	140 nM	0.65
18	17	5.0 nM	240 nM	0.50
54	18	16.6 nM	350 nM	0.70
Family II				
72	103	0.5 nM	270 nM	0.13
72F	21	5.6 nM		
72c	104	4.6 nM	170 nM	0.15
96	22	0.9 nM	130 nM	0.40
79	26	1.7 nM	52 nM	0.35
73t	31	2.4 nM	530 nM	0.40
73tF	32	14.0 nM		
75t	30	0.9 nM	420 nM	0.40
72t.2c	106	5.0 nM	360 nM	0.40
11t	36	0.6 nM	110 nM	0.40
11tF	37	7.0 nM		
4	39	0.7 nM	300 nM	0.55
89	41	2.5 nM	62 nM	0.50
35t	40	2.4 nM	170 nM	0.65
Family III				
9t	45	39.4 nM	310 nM	0.25
6t	46	28.0 nM	1830 nM	0.50
83t	48	10.5 nM	170 nM	0.30
86t	49	2.8 nM	450 nM	0.35
86tF	50	12.0 nM		

Tabl 3 (Pag 2)

Ligand	SEQ ID NO	Kd1	Kd2	Mole fraction
Family V				
80	53	1.6 nM	300 nM	0.40
87	54	0.8 nM	450 nM	0.50
87F	55	1.2 nM	840 nM	0.50
87.3	109	0.8 nM	720 nM	0.40
87.4	110		4350 nM	
87.5	111	3.1 nM	1320 nM	0.50
87.7	112	0.8 nM	250 nM	0.30
A: 2'OH Ligands				
Unrelated Sequence				
1	56	1.4 nM	43 nM	0.25
60	59	0.3 nM	200 nM	0.20
Random RNA				
30N1	1		360 nM	
30N1F	1		35 nM	
B: 2'NH_2 Ligands				
Family VI				
NN11	60	2.8 nM		
NN27	61	0.4 nM	150 nM	0.2
NN16	62	0.9 nM		
NN10	63	0.4 nM		
NN19	64	0.4 nM		
NN2	65	1.2 nM		
NN11d	66			
NN29	67	2.5 nM		
NN41	68	1.2 nM		
NN5	69	4.6 nM		
NN19.4	117	7.3 nM		
NN19.11	118	0.5 nM		
NN19.13	119	2.2 nM		
NN19.14	120	1.0 nM		
NN19.15	121	1.7 nM		
NS2	122	22.0 nM		

Table 3 (Page 3)

Ligand	SEQ ID NO	Kd1	Kd2	Mole fraction
Other Sequences				
NN1	72	21 nM		
NN4	71	27 nM		
NN18	78	71 nM		
NN22	75	36 nM		
NN30	79	23 nM		
NN39	80	52 nM		
NN40	77	31 nM		
NS1	88	42 nM		
NS4	84	24 nM		
NS6	94	28 nM		
A; 2'NH_2 Ligands				
NS7	96	18 nM		
NS10	92	22 nM		
NS11	87	16 nM		
NS12	85	40 nM		
NS13	91	15 nM		
NS14	93	12 nM		
NS18	95	52 nM		
NS39	82	16 nM		
NS48	90	42 nM		
NS49	89	26 nM		
Random 2'NH_2 RNA				
50N7 RNA	4	71 nM		
50N9 RNA	7	48 nM		

Table 4

Selectivity of $sPLA_2$ RNA Ligands

K_d(nM) to specified protein*

Seq. ID#	Ligand	Lysozyme	bFG	$bpPLA_2$	$sPLA_2$
11	2	1,700	230	20,000	1.7
20	72	1,500	180	29,000	0.5
53	80	2,70	240	29,000	1.6
54	87	2,300	220	20,000	0.8
1	30N1	1,650	270	22,500	360

* Standard binding experiments were performed to the specified target protein in TBSC and K_d's calculated by curve fitting. The ligands bind no better than randomized RNA (30N1) to lysozyme (hen egg-white), human basic fibroblast growth factor basic (bFGF) and bovine pancreatic PLA_2. The $sPLA_2$ K_d is that of the high affinity species.

Table 5

Selectivity f $sPLA_2$ 2'NH_2 RNA Ligands

K_d(nM) to specified protein*

Seq. ID#	Ligand		Elastase	bFGF	C1q	IgG	$sPLA_2$
64	NN19	40	210	-	>5,000	1	
121	NN19.15	40	165	1,000	4,300	0.4	
7	50N9	70	170	1,000	>5,00	48	

* Standard binding experiments were performed to the specified target protein in TBSC and K_d's calculated by curve fitting. The ligands bind no better than randomized RNA (30N1) to human neutrophil elastase, human basic fibroblast growth factor (bFGF), and human immunoglobulin G (IgG and C1q).

TABLE 6

Starting RNA:

5'-GGGAGCUCAGAAUAAACGCUCAA[-30N-]UUCGACAUGAGGCCCGGAUCCGGC-3'
(SEQ ID NO:123)

PCR Primer 1:
Hind III

5'-CCGAAGCTTAATACGACTCACTATAGGGAGCTCAGAATAAACGCTCAA-3'
(SEQ ID NO: 124) T7 Promoter

PCR Primer 2:
Bam H1

5'-GCCGGATCCGGGCCTCATGTCGAA-3'
(SEQ ID NO:125)

TABLE 7

SEQ ID NO:	Ligand	SEQUENCE	VEGF Protein Binding Kd	VEGF Receptor Binding Ki
126	NX-107	ACC CUG AUG GUA GAC GCC GGG GUG		1 nM
127	NX-176	ACC CUG AUG GUA GAC GCC GGG GUG	65 nM	10nM
128	NX-178 T*T*T*T*	ACC CUG AUG GUA GAC GCC GGG GUG T*T*T*T*T	0.7 nM	1 nM
129	NX-190 T*T*T*T*	*ACC CTG ATG GTA GAC GTT GGG GTG T*T*T*T*T*		
130	NX-191 T*T*T*T*	ACC CUG AUG GUA GAC GCC GGG GUG T*T*T*T*T	120 nM	500nM
131	NX-213 T*T*T*T*	ACC CUG AUG GUA GAC GCC GGG GUG T*T*T*T*T	0.2 nM	1 nM
132	NX-214 T*T*T*T*	ACC CUG AUG GUA GAC GCC GGG GUG T*T*T*T*T	0.2 nM	1 nM
133	NX-215 T*T*T*T*	ACC CUG A*UG GUA GAC GCC GGG GUG T*T*T*T*T	0.2 nM	1 nM
134	NX203	ACC CUG AUG GUA GAC GCC GGG GUG		
135	NX204	ACC CUG AUG GUA GAC GCC GGG GUG		
136	NX205	ACC CUG AUG GUA GAC GCC GGG GUG		
137	NX206	ACC CUG AUG GUA GAC GCC GGG GUG		

N = 2'OH N = 2'NH_2 N = 2'OMe
N* = phosphorothioate *N* = 2'deoxy
N = 2'OMe:2'OH::2:1

TABLE 8

EX VIVO RAT TISSUE STABILITY: BRAIN

PER CENT FULL LENGTH

TIME (min)	NX 107	NX 178	NX 190	NX191	NX 213
3	94.82	100.48	99.61	98.09	100.33
10	91.66	96.27	99.23	97.75	99.81
30	79.47	86.98	97.53	96.54	99.00
60	73.04	79.39	96.37	95.45	99.02

TABLE 9

EX VIVO RAT TISSUE STABILITY: KIDNEY

PER CENT FULL LENGTH

TIME(min)	NX 107	NX 178	NX 190	NX 191	NX 213
3	90.34	96.07	99.05	97.07	100.01
10	69.97	96.55	97.13	97.76	100.13
30	46.30	92.37	94.56	98.53	99.40
60	45.00	90.14	91.83	97.75	99.09

TABLE 10

Starting Single Stranded DNAs and the Corresponding PCR Primers Used in the ssDNA SELEX Experiments Targeting VEGF

SELEX experiment A

Starting ssDNA:
5'-ATCCGCCTGATTAGCGATACT(40N)ACTTGAGCAAAATCACCTGCAGGGG-3'
(SEQ ID NO: 216)

PCR Primer 1:
5'-JJJCCCCTGCAGGTGATTTTGCTCAAGT-3' (SEQ ID NO: 217)

PCR Primer 2:
5'-ATCCGCCTGATTAGCGATACT-3' (SEQ ID NO: 218)

SELEX Experiment B

Starting ssDNA:
5'-CTACCTACGATCTGACTAGC(40N)GCTTACTCTCATGTAGTTCCT-3'
(SEQ ID NO: 219)

PCR Primer 1:
5'-AJAJAGGAACTACATGAGAGTAAGC-3' (SEQ ID NO: 220)

PCR Primer 2:
5'-CTACCTACGATCTGACTAGC-3' (SEQ ID NO: 221)

J=biotin

Table II - VEGF ssDNA Ligands

ssDNA bulk pool, BH SELEX: 0.44 nM

SEQ ID NO:	Ligand		Kd,nM
Family I			
138	3	acaacggcgtggaagactagagtgcagccgaacgcatcta	
139	5	acgctacaagtccgctgtggtagacaagagtgcaggcaag	
140	9 (3x)	aggcccgtcgaagntagagcgcagggccccaaaataccg	
141	10	gtaccatccacggtttacgtggacaagagggccctggtac	1
142	11	tcactacaagtccgccgtggtagacaagagtgcaggcaag	
143	15	accgctgtgtagttccttaggactagagggccgcctac	0.88
144	21	taggcttgacgtcttctagactagagtgcagtcaaaccc	
145	27	tgcaggtcgacttctagaggatccccgggtaccgagctcga	
146	31	acggtttacgtggacaagagggccctggtac	
147	32 (3x)	ggtggactagaggncagcaaacgatccttggttcgcgtcc	2
148	33	tcaagcactcccgtcttccagacaagagtgcagggcctct	2
149	35	cgtgatggacaagagggccctatccaccggatatccgtc	
150	37	caagcagtgcccgtcttccagacaagagtgcaggcctct	
151	39	tgatccaccgtttatagtccgtggtagacaagagtgcagg	
152	41	aacacacaagaggacagttacaggtaacatccgctcagg	
153	49	agtggcgtctatagacaagagtgcagcccgagtttca	
154	50	ccacaagagggcagcaagtg-tacaactacagcgtccgg	
155	b56(8x)	gcagggccacgtctatttagactagagtgcagtggttc	0.5
156	b69	acggtccaaaggtttcccatccgtggactagagggcacgtgctta	
157	b80	ccgtcgcgtgactataaccacacgcagactagagtgcagggctta	8.1
158	b81**	ccgaatgggctgcgactgcagtggacgtcacgtcgtta	0.3
159	b91**	acgcaagagagtcnccgaatgcagctctcagccgctaaca	
210	Consensus	agacaagagtgcagg	
211		ggactagagggcagt	

TABLE II (PAGE 2)

SEQ ID NO:	Ligand		Kd PCR
	Family 2		
160	2	canncactgcaagcaattgtggcccaaagggctgagt	
161	14	gctcgcttacaaaagggagccactgtagcccagactggac	
162	25 (2x)	ggttatggtgtggttccgaatggtgggcaaagtaacgctt	
163	40	gcttgtngctccgaaggggcgcgtatccaaggacggttc	
164	46	tatggagtggttccgaatggtgggcaaagtaacgctt	
165	b54	tgcnngcgggcggttctccggatgggaccataaggctttagctta	2.5
166	b55	acaaggggtcctgnngaatgggggaatacgctagccgaa	15
167	b59	aacacgagcatgtgggggtcccttccgaatgggggtacaggctta	91
168	b79	gaggcattaggtccgaatggtagtaatgctgtcgtgccttgctta	2
169	b81**	ccgaatgggctgcgactgcagtggacgtcacgtcgtta	0.3
170	b85	gaggaggtgcgttgtccgaaggggtcgttagtcacctcgtgctta	0.15
171	b88 (5x)	gcaaggggtcctgccgaatgggggaatacgctagccgaaa	34
172	b89 (3x)	atccttccgaatgggggaaatggcgnccca	2
173	b91**	acgcaagagaggtcnccgaatggcagtctcagccgctaaca	3.9
174	b99	cacgataatcctccgaaagcgttgtccgaatgggtcgttagctta	34
212	Consensus	ctccgaatgggggnaaa g	
	Family 3		
175	18	tatcacccccactggatagagccgcagcgtgccctact	
176	19	gcccactgcatagagggacggttgtttccgcccggtgttt	
177	b51	gtgaaggagccccaactggatagaagccttaaggcggtgt	
178	b60	ccaccgcagagtgttacacccccataggagaagtccggatggctta	26
179	b62	ccactgcatagagagtcgcaagacacggtgctttattcnccgctta	2.9
180	b63	tgccccactggatagagtaggaggcctagccgacacggtgctta	
181	b65	cgaggtcccccactggatagagttgttgaaacaacggtgcgctta	0.53
182	b66	aacacttccccactggatagaggccttcgcagagccggtgctta	1.3

TABLE 11 (PAGE 3)

SEQ ID NO:	Ligand		Kd PCR
	Family 3 (con't)		
183	b95	ccactgcatagagaactggatcgacggtccaaagttcggtgctta	0.9
184	b96	ccactgcatagagatactggattcgacnnnccaaagtttcggtgctta	1.5
185	b97	ccactgcagagagtcaaccttacgangccaaggttgcggtgctta	>1
213	Consensus	ccccactggatagag	
214		ccccactgcatagag	
	Family 4		
186	1	tctgcgagagacctactggaacgttttgtgatattcaca	12
187	6	atacacccggcgggcctaccggatcgttgatttctctcc	1.0
188	13	acgccccctgagacctaccggaatnttntcgctaggccta	
189	23	gggcatctaacccagacctaccggaacgttatcgcttgtg	0.75
190	44	ggtgtgaaccagacctacnggaacgttatcgcttgtg	0.4
215	Consensus	agacctaccggaacgtt	
	Orphans		
191	4	catcagtattatataacgggaaccaacggcaaatgctgac	
192	7	tccnngggagaatagggttagtcggagaagttaatcgct	
193	16	cgggaacgtgtggttacncggcctactggattgtttcctg	
194	30	ggtaggtccggtgtgaaagaggttcgcatcaggta	
195	38	cctcaggcaacatagttgagcatcgtatcgatcctggag	
196	43	ttggcttgagtcccgggacgcactgttgacagtggagt	
197	45	cagcaggttagtataacgggaaccaacggcaaatgctgac	
198	b53	gcaagggcatctcggaatcggttaatctgacttgcaatacgctta	2.5
199	b98	gatccacgaagaagcttactctcatgtagttcca	>100

TABLE II (PAGE 4)

SEQ ID NO:	Ligand		Kd PCR
	Truncates		
		5	
		gtaccatccacggtttacgtggacaagagggccctggtac	3
		gtagttcctttaggactagagggccgcctac	17
		tggactagaggncagcaaacgatccttggttcgcgtcc	0.7
		cccgtcttccagacaagagtgcaggg	0.2
		agggccacgtcttatttagactagagtgcagtggttc	0.3
		ggaggtgcgttgtccgaagggtcgttagtcacctc	19
		gcaaggggtcctgccgaatgggggaatacgctagccgaaa	0.32
		cgaggtcccccactggatagagttgttgaaacaacggtgcgctta	0.35
		aacacttccccactggatagaggcctttcgcagagccggtgctta	>200
		gggcatctaacccagacctaccggaacgttatcgcttgtg	

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TABLE 12

Starting template sequence for SELEX "A":
5'-GGGAGACAAGAATAAACGCTCAA-50N-TTCGACAGGAGGCTCACAACAGGC-3' (SEQ ID NO:222)

5' PCR primer for SELEX "A":
5'-TAATACGACTCACTATAGGGAGACAAGAATAAACGCTCAA-3'(SEQ ID NO:223)

3' PCR primer for SELEX "A":
5'-GCCTGTTGTGAGCCTCCTGTCGAA-3'(SEQ ID NO:224)

Starting RNA sequence pool for SELEX "A":
5'-GGGAGACAAGAAUAAACGCUCAA-50N-UUCGACAGGAGGCUCACAACAGGC-3' (SEQ ID NO:225)

5' primer for cloning evolved ligands from SELEX "A":
5'-GCCGGATCCGCCTGTTGTGAGCCTCCTGTCGAA-3'(SEQ ID NO:226)

3' primer for cloning evolved ligands from SELEX "A":
5'-CCGAAGCTTAATACGACTCACTATAGGGAGACAAGAATAAACGCTCAA-3' (SEQ ID NO:227)

primer for sequencing cloned ligands from SELEX "A" and "B":
5' TAATACGACTCACTATA 3' (SEQ ID NO:228)

TABLE 13

Evolved gag ligands from SELEX experiment "A"

5'-GGGAGACAAGAAUAAACGCUCAA-50N-UUCGACAGGAGGCUCACAACAGGC-3' (SEQ ID NO:225)

SEQ ID NO:	Ligand Name	Sequence of randomized region
229	SW8.1	UUGUGAUCUACCGUUACCUGACGGACGUGUUUUUACACCAACGAACCUGG
230	SW8.2	UGAACCUCUGAGUUCUUCAUAGAACUGAUAUCUUCAAGAAGGGGUAGAUG
231	SW8.4	CGUCUUCUACAGGGAACCGUGGUGCAUCUGUGAAGUUGUAGAUUCCUAGU
232	SW8.6	GGGUUGAUUACCAAAUCGUAACCUGUACCCUGCCUACACUAGGACAACGG
233	SW8.20	CUAACCGAAGCUCUGAGAAAUAGUUUAUCCAGUGAAUGAAUCCUGAUGGG
234	SW8.24	CGAGCUUUUAGUAAGACUCAUGCCGAGAAAUCGGUCGUGAUGCUGUCGAG
235	SW8.25	GCCCGGGAAUUGCAUGUUGUGCGUGCCGGGAGUCCAAGUCAGCAUCCUCA
236	SW8.27	GGGUUGAUUACCAAAUCGUAACCUGUACCCUGCCUACCCUAGGACAACGA
237	SW8.30*	AUCCCACUCGGUCGUGACCUGACGUGAAAACGGAUAGGAUCGCACGUCAA
238	SW8.31	GUGUCCCUUGUCACCUGGGACUGGGCCGUUUGAACUGACAUUCUAUACGA
239	SW8.37	GGCCAACGUCCGAGUUGGUUUCCCAUAGCUGAGCACAGGACGGCUUCUGC
240	SW8.40	CCCGGAUGGUGAGCCCUGUCGGAGAUUGGCACGAGGGUAAAGGGUAGGGA
241	SW10.7	CUGUUGCUAAGUAGAAGUCAUAUUCUGCGAUGGUUAAAGAUAACCCAGCC
242	SW10.12	UCUCGUAUCAGCACACCGGUACAAAGAGGAUGCAAAUACGCCUGUGAC
243	SW10.22	CGUCUUCUACAGGGAACCGUGGUGCAUCUGUGAAGUUGUUGUAGAUUCCUAGA
244	SW10.25	UGUCUUGGCGUCCACGUCGUAGUGUGUGGGGGGAAAAGAGGAGGGUGCAC
245	SW10.28	UUGUUAGCCUGGUGAAGCAUGUACUGCGAAGUGGAGGGAAUGUGGAAGGG
246	SW10.34	GCUCCCCUAGCCGACUUCCUGUUGGACGAGGGUGGUACCGUGGGAGGAUG
247	SW10.39	CGAGCCUUUAGUAAGACUCAUGCCGAGAAAUCGGUCGUGAUGCUGUCGAG
248	SW10.43	UUCGUGAGGGCGGUGUGGGAGGCAAGCGGUACGAGCGUACUGUCUGGGCC

* The 5'-fixed region is missing nucleotide A in the 9th position

TABLE 14

Starting template sequence for SELEX "B":

5'-GGGAAAAGCGAATCATACACAAGA-50N-GCTCCGCCAGAGACCAACCGAGAA-3'(SEQ ID NO:249)

5' PCR primer for SELEX "B":

5'-TAATACGACTCACTATAGGGAAAAGCGAATCATACACAAGA-3'(SEQ ID NO:250)

3' PCR primer for SELEX "B":

5'-TTCTCGGTTGGTCTCTGGCGGAGC-3'(SEQ ID NO:251)

Starting sequence of RNA pool for SELEX "B":

5'-GGGAAAAGCGAAUCAUACACAAGA-50N-GCUCCGCCAGAGACCAACCGAGAA-3'(SEQ ID NO:252)

5' primer for subcloning evolved ligands from SELEX "B":

5'-CGCGGATCCTAATACGACTCACTATAGGGAAAAGCGAATCATACACAAGA-3' (SEQ ID NO:253)

3' primer for subcloning evolved ligands from SELEX "B":

5'-GGCGAATTCTTCTCGGTTGGTCTCTGGCGGAGC-3' (SEQ ID NO:254)

TABLE 15

Evolved gag ligands from SELEX experiment "B"

5'-GGGAAAAGCGAAUCAUACACAAGA-50N-GCUCCGCCAGAGACCAACCGAGAA-3' (SEQ ID NO:252)

SEQ ID NO:	Ligand Name	Sequence of randomized region
255	ML8.7	UGUGAUGGGUGUGUUUUGGUUAGCUGUGGAGGGCCAUGUGGGCUGGACG
256	ML8.11	GGUGUGUGUGCGCCGCGAGGUGUCUGAGGAGAGAGGUGGCAGUGGAGGGU
257	ML8.12	GGAGGGUGUGCGGACGGGAGCGUGUAGUGAGGCUUUUCAGGCGUUGGACG
258	ML8.14	UGUGAUGGGUGUGAUUUUGGUUAGCUGUGGAGGGUUAUGUGGGUCGGACG
259	ML8.16	UGUGGGCUUCCUGAGGGGUAGGAACUCUGAAGUCAUGGUUCGUGGUAAGC
260	ML8.17	GGAGGGAAUGUGGAAGGGUUUGUGGUGUUUCGCAAAUGCCGCAUGGACGU
261	ML8.18	GGGGGGAGAGGCGUGGACGAUGUUGUGGUUAUGCUGUCGGUUUUUGGCUUG
262	ML8.19	GGGAUGGAUCGGUGAGACGAGCAGUGGAGUGGUGAGGUGUGGUGUCACGU
263	ML8.20	CGUUCGGUGGUGGACAGGGUAAUGUGGAGGGACCGGGUGAUUGUGUAUGU
264	ML8.21	UGGAGGGUGGCGGGAGAAGUUUGAGGUCGGGGUCGUAUGAUGUGCGCUAG
265	ML8.22	UCACGACGGAGGGCGGAUGAAGGGGGGAAGGUCGUGAGUCCAUGCCGUGU
266	ML8.23	GGAUGGGCGUGAAUGGAGGGCAAUGUGUGGGUUUGUUGAGCCAGUUGGU
267	ML8.24	ACGGGUCCCUAAGACGUCGUUUCGAGAUGUGUGUUUCGGAAAGAGUAGCG

TABLE 16

Two point binding data for ligands from gag SELEX "A"

Ligand	% Ligand bound at 10 nM gag	% Ligand bound at 0.1 nM gag	% Ligand bound at 0.0 nM gag
SW8.1	30	6.6	3.2
SW8.2	7.3	2.9	1.9
SW8.4	27	2	1.3
SW8.6	4.1	1.7	1.5
SW8.20	16	4.1	4.7
SW8.24	20	4.5	2.6
SW8.25	11	1.1	1.0
SW8.27	32	4.6	5.3
SW8.30	3.7	0.8	0.8
SW8.31	6.3	2.4	2.7
SW8.37	4.2	1.8	1.0
SW8.40	15	8.5	3.8
SW10.7	8.6	3.5	3.0
SW10.12	11.2	0.6	0.6
SW10.22	33	3.1	1.7
SW10.25	19	10	13
SW10.28	33	31	16
SW10.34	11.8	7.9	8.2
SW10.39	17	0.8	0.6
SW10.43	6.7	4.1	3.0
Ψ-456	87	13.4	14.7
Round 0 RNA	3.5	4.0	6.0

Table 17. Affinity of nucleic acid ligands for the HIV-1 gag protein.

Sequence ID no.	Ligand name	Kd for GST-gag
231	SW8.4	1 nM
234	SW8.24	5 nM
235	SW8.25	5 nM
236	SW8.27	3 nM
238	SW8.31	41 nM
239	SW8.37	50 nM
242	SW10.12	30 nM
245	SW10.28	8 nM
255	ML8.7	12 nM
258	ML8.14	13 nM
263	ML8.20	10 nM
265	ML8.22	10 nM
268	ML6.1	2 nM
271	ML6.6	11 nM
272	ML6.8	3 nM
273	ML6.9	7 nM
276	ML6.17	4 nM

Table 18. Binding specificity of HIV-1 gag nucleic acid ligands

Sequence ID no.	Ligand Name	Binds to[a]	Frequency[b]
230	SW8.2	matrix	1
231	SW8.4	nucleocapsid	12
232	SW8.6	matrix	1
234	SW8.24	nucleocapsid	2
235	SW8.25	nucleocapsid	1
236	SW8.27	matrix	3
237	SW8.30	matrix	1
238	SW8.31	nucleocapsid	1
239	SW8.37	nucleocapsid	1
242	SW10.12	matrix	1
243	SW10.22	nucleocapsid	1
245	SW10.28	nucleocapsid	1
247	SW10.39	nucleocapsid	1
255	ML8.7	matrix	7
256	ML8.11	GST	1
257	ML8.12	filter	1
258	ML8.14	matrix	2
259	ML8.16	filter	1
260	ML8.17	filter	1
261	ML8.18	filter	1
262	ML8.19	filter	1
263	ML8.20	nucleocapsid	2
264	ML8.21	filter	1
265	ML8.22	nucleocapsid	1
266	ML8.23	filter	1
267	ML8.24	filter	1

Table 18 (page 2)

Sequence ID no.	Ligand Name	Sequence	Binds to	Frequency[b]
268	ML6.1	ACAGCGAUUUGUGUGCUCGUCGGCUGUUUGCAGAUGUAUGGAACCAUGC	nucleocapsid	1
269	ML6.2	GGGCUUGGGGUGGGGUCUUGCAGACGGAUAUGGUAAGCUGCUGCUGGCU	GST-gag	1
270	ML6.3	AUUGGGAACGUGGCGCAUCGGGAGUGUAUGGUGGCUUGUGGGCGGGUUA	GST-gag	1
271	ML6.6	AGCCAGGUCUUCAAUGGUAGUUGUUGAUUGGUGGUGCUUUGCAGAUGUAU	nucleocapsid	1
272	ML6.8	UCGGUUAUGGAGAGGCGGUAGAGAGCGAGUAGCAAGUAACGGUGCAAAAG	matrix	1
273	ML6.9	CCGCACUUCGAUUGCAUUGUCGAGGAGAGCGGGUGCAGAUGUACGGGUUUG	nucleocapsid	1
274	ML6.12	AGACUUGGUACGCGUGGUUAGCUAGGAGGGGGGGAGAAGAUAAGGCGAUAG	GST-gag	1
275	ML6.16	GAGUUUUGCUUCGACAUGGGGUUAGCUGUGGAGCAGGGUGACCUGGGACC	GST-gag	1
276	ML6.17	UGGAGUGGGUAGGCGUGGGAGGUAUGGCAUCUUGUGUGUGCCAAGAUGGU	?	1

[a] Filter means the nucleic acid ligand binds to nitrocellulose filters and not to the GST-p55 (HIV-1 gag) protein used in the SELEX procedure. GST-gag means the nucleic acid ligand binds to the GST-p55 (HIV-1 gag) protein used in the SELEX procedure, but that binding to individual components of the HIV-1 gag polyprotein has not been determined. Nucleocapsid, matrix, and GST mean the nucleic acid ligand binds to the GST-p7 (HIV-1 nucleocapsid) protein, the HIV-1 p17 matrix protein, or the glutathione S-transferase protein described in Materials and Methods, respectively. A question marks indicates the nucleic acid ligand binds to the GST-p55 (HIV-1 gag) protein, but does not bind to GST, GST-p7 (HIV-1 nucleocapsid), GST-p15 (HIV-1 nucleocapsid p7-p6), p17 HIV-1 matrix, or p24 HIV-1 capsid proteins.

[b] Frequency indicates the number of clones sequenced for each nucleic acid ligand at the end of the SELEX procedure.

Table 19. Boundaries of HIV-1 gag nucleic acid ligands.

Sequence ID no.	Ligand Name[a]	Boundary[b]
SELEX "A" fixed region		GGGAAAAGCGAAUCAUACACAAGA ... GCUCCGCCAGAGACCAACCGAGAA
268	ML6.1$_{NC}$	GGGAAAAGCGAA**UCAUACACAAGAACAGUGAUUUGUGUGCUCGUCGGCUGUUugcgaguguaUGGAACCAUGCAGCUCCGCCAGAGACCAACCGAGAA** (5' … 3')
271	ML6.6$_{NC}$	GGGAAAAGCGAAUC**AUACACAAGAAGCCAGGUCUUCAAUGGUAGUUGUUGAUUGGUGGUGCUUugcagauguaUGCUCCGCCAGAGACCAACCGAGAA** (5' … 3')
272	ML6.8$_{NA}$	GGGAAAAGCGAAUCAUACACAAGAUCGGUUAUGGAG**AGGCGGUAGAGAGCGAGUAGCAAGUAACGGUGCAAAAGGCUCC**GCCAGAGACCAACCGAGAA (5' … 3')
273	ML6.9$_{NC}$	GGGAAAAGCGAAUCAUACACAAGACCGCA**CUUCGAUUGCAUUGUCGAGGAGAGCGGgugcagaugu**acGGGUUUGGCUCCGCCAGAGACCAACCGAGAA (5' … 3')
255	ML8.7$_{MA}$	GGGAAAAGCGAAUCAUACACAAGAUGUGAUGGGUGUGAUUUU**GGUUAGCUGUGGAGGGCCAUGUGGGCUGGA**CGGCUCCGCCAGAGACCAACCGAGAA (5' … 3')
263	ML8.20$_{NC}$	GGGAAAAGCGAAUCAUAC**ACAAGACGUUCGGUGGUGGACAGGGUAAUGUGGAGGGACCGGGUGAUuguguaugug**CUCCGCCAGAGACCAACCGAGAA (5' … 3')
265	ML8.22$_{NC}$	GGGAAAAGCGAAUCAUACACAAGAUCACGACGGA**GGGCGGAUGAAGGGGGGAAGGUCGUGAGUCcaugccgugug**CUCCGCCAGAGACCAACCGAGAA (5' … 3')
SELEX "B" fixed region		GGGAGACAAGAAUAAACGCUCAA ... UUCGACAGGAGGCUCACAACAGGC
231	SW8.4$_{NC}$	GGGAGACAAGAAUAAACGCUCAACGUCUUCUACA**GGGAACCGUgguugcaucU**GUGAAGUUGUAGAUUCCUAGUUUCGACAGGAGGCUCACAACAGGC (5' … 3')
234	SW8.24$_{NC}$	GGGAGACAAGAAUAAACGCUCAACGA**GCUUUUAGUAAGACUCAUGCCGAGAAAUCGGUCGUGAUGcugucgaguucgacag**GAGGCUCACAACAGGC (5' … 3')
	SW8.25$_{NC}$	GGGAGACAAGAAUAAA**CGCUCAAGcccggg**AAUUGCAUGUUGUGCGUGCCGGGAGUCCAAGUCAGCAUCCUCAUUCGACAGGAGGCUCACAACAGGC (5' … 3')
242	SW10.12$_{MA}$	GGGAGACAAGAAUAAACGCUCAAUCUCGU**AUCAGCACACCGGUACAAAGAGGAUGC**AAAUACGCCUGUGACCUUCGACAGGAGGCUCACAACAGGC (5' … 3')
245	SW10.28$_{NC}$	GGGAGACAAGAAUAAACGCUCAAUUGuua**gccuggugaaGCAUGUACUGCGAAGUGGAGGGA**AUGUGGAAGGGUUCGACAGGAGGCUCACAACAGGC (5' … 3')

[a] A NC suffix indicates a HIV-1 nucleocapsid nucleic acid ligand. A MA suffix indicates a HIV-1 matrix nucleic acid ligand.

[b] 5' indicates the position of the 5' nucleic acid ligand boundary. 3' indicates the position of the 3' nucleic acid ligand boundary. Underlined regions designate the minimal nucleic acid ligand sequence that is predicted to bind to the HIV-1 gag protein, based on the position of the 5' and 3' boundaries. Lower case lettering indicates a palindrome motif found in nucleocapsid ligands. The sequences of the fixed regions used in SELEX procedure "A" and "B" are indicated. The sequences evolved during the SELEX proceedure are located between the fixed sequences.

Table 20. Binding properties of truncated HIV-1 gag nucleic acid ligands.

Sequence ID no.	Ligand Name[a]	Sequence of truncated nucleic acid ligand[b]	Binding[c]
277	ML6.1_{NC}t13-63	gggUCAUACACAAGAACAGUGAUUUGUGUGCUCGUCGGCUGUUUGCGAGUGUAU	+
278	ML6.6_{NC}t15-74	gggAUACACAAGAAGCCAGGUCUUCAAUGGUAGUUGUUGAUUGGUGGUGCUUUGCAGAUGUAU	+
279	ML6.8_{MA}t33-82	gGGAGAGGCGGUAGAGAGCGAGUAGCAAGUAACGGUGCAAAAGGCUCCGCC	+
280	ML8.7_{MA}t40-72	GggUUUGGUUAGCUGUGGAGGGCCAUGAGGGCUGGA	-
281	ML8.20_{NC}t14-73	gggCAUACACAAGACGUUCGGUGGUGGACAGGGUAAUGUGGAGGGACCGGGUGAUUGUGUAUGUG	+
282	ML8.22_{NC}t23-79	gGAUCACGACGGAGGGCGGAUGAAGGGGGGAAGGUCGUGAGUCCAUGCCGUGUGCUCC	+
283	SW8.4_{NC}t41-55	gggCGUGGUGCAUCUGUGccc	-
284	SW8.4_{NC}t30-53	GggagCUACAGGGAACCGUGGUGCAUCUG	+
285	SW10.12_{MA}t28-56	gggGUAUCAGCACACCGGUACAAAGAGGAUGC	-

[a] The name of the full length nucleic acid ligand from which the truncate is derived is indciacted, beginning with the letters ML or SW. The NC suffix indicates a truncation of the indicated HIV-1 nucleocapsid nucleic acid ligand. The MA suffix indicates a truncation of the indicated HIV-1 matrix nucleic acid nucleic acid ligand. The lower case "t" designates the RNA as a truncated nucleic acid ligand. The numbers following the "t" indicate the nucleotide positions from the full length nucleic acid ligand that are included in the truncated nucleic acid ligand.

[b] Lower case letters are nucleotides added to facilitate generation of the truncated nucleic acid ligand by transcription or, in the case of SW8.4 to promote stem/loop formation, but are not found in the full length nucleic acid ligand sequence.

[c] A plus indicates the truncated nucleic acid ligand binds to the appropriate HIV-1 protein at least 10 fold better than random RNA. A minus indicates the truncated nucleic acid ligand does not bind to the appropriate HIV-1 protein any better than random RNA.

Table 21. Binding properties of mutant HIV-1 gag nucleic acid ligands.

Sequence ID no.	Ligand Name[a]	Sequence of mutant nucleic acid ligand	Binding[b]
286	ML6.8$_{MA}$(D56-57)	GGGAAAAGCGAAUCAUACACAAGAUCGGUUAUGGAGAGGCGGUAGAGAGCGAGUAAAGUAACGGUGCAAAAGGCUCCGCCAGAGACCAACCGAGAA	-
287	SW8.4$_{NC}$(G47C)	GGGAGACAAGAAUAAACGCUCAACGUCUUCUACAGGGAACCGUGGUCCAUCUGUGAAGUUGUAGAUUCCUAGUUUCGACAGGAGGCUCACAACAGGC	-
288	SW8.4$_{NC}$ (GUCCAU45-50GCGCGC)	GGGAGACAAGAAUAAACGCUCAACGUCUUCUACAGGGAACCGUGGCGCGCCUGUGAAGUUGUAGAUUCCUAGUUUCGACAGGAGGCUCACAACAGGC	-

[a] The name of the nucleic acid ligand that was mutated is indicated. The NC suffix indicates a mutant of the indicated HIV-1 nucleocapsid nucleic acid ligand. The MA suffix indicates a mutant of the indicated HIV-1 matrix nucleic acid nucleic acid ligand. The numbers and letters in parentheses following the nucleic acid ligand name indicate which nucleotides were changed, what their positions are, and what sequence they were changed to within the mutated nucleic acid ligand. Further details are described in the text. A "D" sign indicates nucleotides that were deleted.

[b] A minus indicates the mutated nucleic acid ligand does not bind to the appropriate HIV-1 protein any better than random RNA.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Larry Gold
David Parma
Nebojsa Janjic
Michael Lochrie

(ii) TITLE OF INVENTION: High-Affinity Nucleic Acid Ligands To Secretory Phospholipase A_2 ($sPLA_2$), Vascular Endothelial Growth Factor (VEGF), and Human Immunodeficiency Virus type-1 GAG (HIV-1 GAG)

(iii) NUMBER OF SEQUENCES: 289

(iv) CORRESPONDENCE ADDRESS:
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(v) COMPUTER READABLE FORM:
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(B) COMPUTER: IBM compatible
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WordPerfect 6.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US96/02942
(B) FILING DATE: 04 MAR 96

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/447,169
(B) FILING DATE: 19 MAY 1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/447,172
(B) FILING DATE: 19 MAY 1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/399,412
(B) FILING DATE: 06 MARCH 1995

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/233,012
(B) FILING DATE: 25 APRIL 1994

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/205,515
(B) FILING DATE: 03 MARCH 1994

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/117,991
(B) FILING DATE: 08 SEPTEMBER 1993

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/964,624
(B) FILING DATE: 21 OCTOBER 1992

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/931,473
(B) FILING DATE: 17 AUGUST 1992

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/714,131
(B) FILING DATE: 10 JUNE 1991

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 07/536,428
(B) FILING DATE: 11 JUNE 1991

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(A) TELEPHONE: (303) 793-3333
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(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGGAGCUCAG AAUAAACGCU CAANNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 46 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:
CCGAAGCTTA ATACGACTCT ATAGGGAGCT CAGAATAAAC GCTCAA 46

(2) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
GCCGGATCCG GGCCTCATGT CGAA 24

(2) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
GGGAGGACGA UGCGGNNNNN NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN NNNNNCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
TAATACGACT CACTATAGGG AGGACGATGC GG 32

(2) INFORMATION FOR SEQ ID NO:6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCGGGCGAGT CGTCCTG 17

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 98 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGGAAAAGCG AAUCAUACAC AAGANNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN NNNNNNNNNN NNNNGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TAATACGACT CACTATAGGG AAAAGCGAAT CATACACAAG A 41

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTCTCGGTTG GTCTCTGGCG GAGC 24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 76 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGGAGCUCAG AAUAAACGCU CAAUCUCAUG CUCGUCGCAC GGCGUAACCU 50
AUUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:11:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
GGGAGCUCAG AAUAAACGCU CAAUCUCAUU GCUCGUCGCA CGGCGUAACC 50
UAUUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:12:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:
GGGAGCUCAG AAUAAACGCU CAAAGCUCAU CGUCUCGCAA GGCGUAUCCU 50
AUUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
GGGAGCUCAG AAUAAACGCU CAAACCUCGC CUAUGUUCGC GCGGCGUAUC 50
CUAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
GGGAGCUCAG AAUAAACGCU CAACAGCCAA UGUGUCCCGU ACGGCGUAUC 50
CUAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGGAGCUCAG AAUAAACGCU CAACGCUGGA UACCAUCGCA CGGCGUAUCC 50
UGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GGGAGCUCAG AAUAAACGCU CAAAUUGCAU CAUGUACCGC AAGACGUAUU 50
CUAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GGGAGCUCAG AAUAAACGCU CAAAUCGCAU CAUGUACCGC AAGACGUAUU 50
CUAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GGGAGCUCAG AAUAAACGCU CAAAUUGCAU CAUGUACCGU AAGACGUAUU 50
CUAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GGGAGCUCAG AAUAAACGCU CAAAUUGCAU CAUGUACCGC AAGACGUAUC 50
CUAUUUCGAC AUGAGGCCCG GAUCCGGC 78

(2) INFORMATION FOR SEQ ID NO:20:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCC CGGCUGAGAC 50
ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCC CGGCUGAGAC 50
ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGUU CGGCUGAGAC 50
ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGGAGCUCAG AAUAAACGCU CAAUGCCUCU GCUUACGGGU AAUGCCGAGA 50
CACUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGGAGCUCAG AAUAAACGCU CAAAGUCCUC UCCUUACGGU UCGCCCGAGA 50
UAAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCC CGGCUGAGAC 50
AUUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:26
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCU CGGCUGAGAC 50
ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGUC CGGCUGAGAC 50
ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGGAGCUCAG AAUAAACGCU CAAGACCUCU GCUUACAGCC CGGCUGAGAC 50
GCUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
GGGAGCUCAG AAUAAACGCU CAAGGCCUCU GCUUACGGCU AAUGCCGAGA 50
CGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
GGGAGCUCAG AAUAAACGCU CAAGANCUCU GCUUACAGCC CGGCUGGGAC 50
ACUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:
GGGAGCUCAG AAUAAACGCU CAACUUACAG UUCGGCUGAG AGAAGACGCA 50
UACUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:
GGGAGCUCAG AAUAAACGCU CAACUUACAG UUCGGCUGAG AGAAGACGCA 50
UACUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:33:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GGGAGCUCAG AAUAAACGCU CAACUUACAG UUCGGCUGAG ACGAAGAUCG 50
ACCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GGGAGCUCAG AAUAAACGCU CAACUUACAG GAGAUUCCAU CUCGCUGAGA 50
CGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GGGAGCUCAG AAUAAACGCU CAACUUACGG CAGCGAUUGC UGGCCGAGAA 50
ACCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGGAGCUCAG AAUAAACGCU CAACUUACGG GUAAAGCCGA GAAAAUGUAU 50
UGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine

(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:
GGGAGCUCAG AAUAAACGCU CAACUUACGG GUAAAGCCGA GAAAAUGUAU 50
UGCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:38:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:
GGGAGCUCAG AAUAAACGCU CAAUUGUCUU ACAGGUAAAG CUGAGGAAUC 50
GUUUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:39:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:
GGGAGCUCAG AAUAAACGCU CAAUGUCUUA CGGGUAAAGC CGAGAAAGUU 50
UCCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:40:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:
GGGAGCUCAG AAUAAACGCU CAAGGCUGGG UCUUUUACAG GUAAAGCUGA 50
GAAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO: 41:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:
GGGAGCUCAG AAUAAACGCU CAAAGUCUUA CGGGUAAAGC CGAGAAAGUU 50
UCCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:42:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:
GGGAGCUCAG AAUAAACGCU CAAUCAUGUC AUUACGGGUA AAGCCGAGUU 50
UCUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:43:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:
GGGAGCUCAG AAUAAACGCU CAAAUCAUGU CAUUACGGGU AAAGCCGAGU 50
UUCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:44:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:
GGGAGCUCAG AAUAAACGCU CAACACGAGG GUGGGUGGGU GGCCGAGCGC 50
UUGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:45:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:
GGGAGCUCAG AAUAAACGCU CAACACGGGG GUGGGUGGGU GGCCGAGCGC 50
UUGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:46:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GGGAGCUCAG AAUAAACGCU CAAUGCCUCA UGCCAAUGUG GGAGGGUGGG 50
UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GGGAGCUCAG AAUAAACGCU CAACGCCUCA UGCCAACGCG GGAGGGUGGG 50
UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

GGGAGCUCAG AAUAAACGCU CAACGCCUCA UGCCAACGUG GGAGCGUGGG 50
UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GGGAGCUCAG AAUAAACGCU CAACGCCUCA UGCCAAUGCG GGAGGGUGGG 50
UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GGGAGCUCAG AAUAAACGCU CAACGCCUCA UGCCAAUGCG GGAGGGUGGG 50

UGGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:51:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:
GGGAGCUCAG AAUAAACGCU CAAUCCGGGA GCUGAAAAAC AUGCCGUUAG 50
CCGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:52:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:
GGGAGCUCAG AAUAAACGCU CAAUCCGCGA GCUGAAAAAC AUGCCGUUAG 50
CCAUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:53:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:
GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC AGUGUCCAGC 50
AUCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:54:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:
GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:55:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-F cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-F uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:56:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

GGGAGCUCAG AAUAAACGCU CAAGCCGAAC CGAAUGGAGG UGGAGGGAUU 50
GCGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:57:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

GGGAGCUCAG AAUAAACGCU CAAGACCACG UCCGAACGAA CACCGCCACG 50
CAUUCGACAU GAGGCCCGGA UCCGGC 76

(2) INFORMATION FOR SEQ ID NO:58:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GGGAGCUCAG AAUAAACGCU CAACCAACGA CACUCACGCA UUGCCCACGA 50
ACGUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:59:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 78 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGGAGCUCAG AAUAAACGCU CAAACAACGG CCCACACGGG AGAUCCGAGA 50
AAAGUUCGAC AUGAGGCCCG GAUCCGGC 78

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC GGGGAAACCC GAGGUCCGAG 50
GUAACGCAUG GCGCCUCACC GAGUCGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC GGGGAAACCC GAGGUCCGAU 50
GUAACGCAUG GCGCCUCACC GAGUCGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC GGGGAAACCC GAGAUCCGAG 50
GUAACGCAUG GCGCCUCACC GAGUCGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:63:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC GGCGCCAUAG CCGAGAUCCG 50
AGGUUGUACG AUGACAACUC AGUGCUCCGC CAGAGACCAA CCGAGAA 97

(2) INFORMATION FOR SEQ ID NO:64:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC GGCGCCAUAG CCGAGAUCCG 50
AGGUGUUGAA CGAUAACUCG GUGCUCCGCC AGAGACCAAC CGAGAA 96

(2) INFORMATION FOR SEQ ID NO:65:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

GGGAAAAGCG AAUCAUACAC AAGAGACCGG CCAGCCAAGG CGCUGAGAUC 50
CGAGGUUUCA GAACCCAUCG GGUUGGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:66:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC CGGUAUGUAG CCGGAGAUCC 50
GAGACUUGCU GAACGAGGUG CCACGGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:67:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC CGGUGUGUAG CCGGAGAUCC 50
GAGACUUGCU GAACGAGGUG CCACGGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:68:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

GGGAAAAGCG AAUCAUACAC AAGACCGGCC CGGUGUGCAG CCGGAGAUCC 50
GAGACUUGCU GAACAAGGUG CCACGGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:69:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:
GGGAAAAGCG AAUCAUACAC AAGACCGGCC CCGCCAAUCA AGGGAGAUCC 50
GAGGAAUUGG AAUGUUUGUG AGUGAGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:70:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:
GGGAAAAGCG AAUCAUACAC AAGAGUGUGC GGUGCAUGCG UGGUGAAAGG 50
GGGGUGGGGA AGAAAAACCG GCCCGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:71:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:
GGGAAAAGCG AAUCAUACAC AAGAGUGUGC GGUGCAUGCG UGGUGAAAGG 50
UGGGUUGUGG AGGAAGACCG UGCCGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:72:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:
GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAUGCGGCG AGUGGAGGGG 50

GACUGAUGGA GGGCGAGACC GUGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:73:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:
GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAGGCGGCG AGUGGAGGAG 50
GACUGAUGGA GGGCGAGACC GCAGGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:74:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:
GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAAGCGGCG AGUGGAGGAG 50
GACUGAUGGA GGGCGAGACC GCAAGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:75:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:
GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAUGCGGCG AGUGGAGGAG 50
GACUGAUGGA GGGCGAGACC GUGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:76:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

GGGAAAAGCG AAUCAUACAC AAGACGGCGA GCAGGCGGCG AGUGGAGGAG 50
GACUGAUGGA GGGCGAGACC GCGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:77:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

GGGAAAAGCG AAUCAUACAC AAGACCCCUU GAGCUCGUGA CGCAGGAGGA 50
GGGCCGAGGA GGAAAGUCGU CACAGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:78:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GGGAAAAGCG AAUCAUACAC AAGAGGAUGG CGGCAAGGCG CGAAAGGGAG 50
GAUCGAGGAG GAAUCGCGUC AGGAGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:79:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

GGGAAAAGCG AAUCAUACAC AAGAGCCAGC GAGUGUCGAC AGUGUGGGUG 50
GAAGUGACGG GAGGAUUGGA GACGCUCCGC CAGAGACCAA CCGAGAA 97

(2) INFORMATION FOR SEQ ID NO:80:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GGGAAAAGCG AAUCAUACAC AAGACUGGUU GUGCGGACCC AGUGAGUGGG 50
AGGACGUGAG GGUGGCAGCU GGGCUCCGCC AGAGACCAAC CGAGAA 96

(2) INFORMATION FOR SEQ ID NO:81:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GGGAGGACGA UGCGGAGGGU GGAUCGUGGA GGAAAAGCAU CGUGUGUAAC 50
CGAACCGAUC GUGGYCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:82:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

GGGAGGACGA UGCGGGUAGG GAUGAAGUGC GAUGUGAAUC CGGGUGCUAG 50
CGAUGAUGUG UGCCCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

GGGAGGACGA UGCGGGUAGG GAGACAGACA CACACGCGGA AAGUAGAGCC 50
AUCGUAACAU GCCCCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GGGAGGACGA UGCGGGUAGG GAUAAGCGAG UGUACAGCGA AUACGACUCG 50
GAAUGCUUGG UGCGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

GGGAGGACGA UGCGGUGAAA GAGAAAGGUU GAGAUGAUUA CAAGCGAAUU 50
GGAUAAGUGU CUGGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:86:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:
GGGAGGACGA UGCGGUGAAA UGAGAAAUGG AUUGAUGAUG AUUACAAGAG 50
AAUUGGAUAA GUGUCUGGUC AGACGACUCG CCCGA 85

(2) INFORMATION FOR SEQ ID NO:87:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:
GGGAGGACGA UGCGGGAGGG AAGGGUGGAA CGGAACUCCG AUAAAGCUGU 50
ACAAGUACGU GGGGUCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:88:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:
GGGAGGACGA UGCGGAUAAG GAGGAGCAAG CGAGAAAUUG AGAAGUAACA 50
AGAUCGACAU GGCCCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:89:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GGGAGGACGA UGCGGAUAAG GAUAAGAUCG AACGAGAGUG AACAAAGUUA 50
AAUACAGUCU GGGGGCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:90:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GGGAGGACGA UGCGGGCUAA GGGAAGACAA UGAGAUAGCA GACAAUCAAC 50
UACACCCAUG UGCGUCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:91:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

GGGAGGACGA UGCGGUGAGC UUAGGAUAGG AGCAACAAGU AGAGUAGAGU 50
GAUAACUAGG GUGGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:92:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:
GGGAGGACGA UGCGGUGACA AAUGAGCAAG UAGCGAUAGA UGUGAUGGAC 50
AGAGACAGCC GGGGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:93:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:
GGGAGGACGA UGCGGAAAUG UGUUAGUGAA UGAUUGAGAG AAGAUAGAUG 50
AUGUUGAAGU CUGGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:94:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:
GGGAGGACGA UGCGGAACUA AAAGACAGAG AGAAAACGAC AAUACGAAGU 50
ACAUAUAACC CUGGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:95:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:
GGGAGGACGA UGCGGAAGUA GAUGAUGGAU UGAGAUGUAA GUGUCAGUAU 50

GAAGAGUCUC UGGGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:96:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:
GGGAGGACGA UGCGGAGGAA AUGAAGUAGU GAGAGUAUAA CAUGAUUAUG 50
AAUACGUGAU GUGGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:97:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:
YMUCAUGUHY CGYAMGRCGU AUYCUAU 27

(2) INFORMATION FOR SEQ ID NO:98:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:
CUCWRCUUAC RGBYMVGCYG AGA 23

(2) INFORMATION FOR SEQ ID NO:99:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:
MAYGNGGGWG GGUGGGUGG 19

(2) INFORMATION FOR SEQ ID NO:100:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:
RAGACCGGCC NGSNNNNNNN SCNGAGAUCC GAGG 34

(2) INFORMATION FOR SEQ ID NO:101:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:
CUCAUUGCUC GUCGCACGGC GUAACCUAUU UCGACAUGA 39

(2) INFORMATION FOR SEQ ID NO:102:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:
GGUCUCAUUG CUCGUCGCAC GGCGUAACCU AUUUCGACAU GAG 43

(2) INFORMATION FOR SEQ ID NO:103:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:
AGACCUCUGC UUACAGCCCG GCUGAGACAC UUCGACAUGA GG 42

(2) INFORMATION FOR SEQ ID NO:104:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:
UAAACGCUCA ACUUACAGUU CGGCUGAGAC GAAGAUCGAC CUUCGACA 48

(2) INFORMATION FOR SEQ ID NO:105:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:
GGGAAGACCU CUGCUUACAG CCCGGCUGAG ACACUUCGAC AUGAGGCC 48

(2) INFORMATION FOR SEQ ID NO:106:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:
GGGAUAAACG CUCAACUUAC AGUUCGGCUG AGACGAAGAU CGACCUUCGA 50
CAU 53

(2) INFORMATION FOR SEQ ID NO:107:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:
GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC AGUGUCCAGC 50
AUCUUCGAC 59

(2) INFORMATION FOR SEQ ID NO:108:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 59 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:
GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGAC 59

(2) INFORMATION FOR SEQ ID NO:109:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 62 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGACA UG 62

(2) INFORMATION FOR SEQ ID NO:110:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 61 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:
GGAGCUCAGA AUAAACGCUC AAGCUCUGGG AUGAUGCCCA CUGUCCAGCA 50
UCUUCGACAU G 61

(2) INFORMATION FOR SEQ ID NO:111:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:
GGGAGCUAUA AACGCAGCUC UGGGAUGAUG CCCACUGUCC AGCAUCUUCG 50
ACAUG 55

(2) INFORMATION FOR SEQ ID NO:112:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 83 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:
GGGAGCUCAG AAUAAACGCU CAAGCUCUGG GAUGAUGCCC ACUGUCCAGC 50
AUCUUCGACA UGUGUAGCUA AACAGCUUUA GGA 83

(2) INFORMATION FOR SEQ ID NO:113:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:
AAGACCGGCC GGGGAAACCC GAGGUCCGAG GUAACGCA 38

(2) INFORMATION FOR SEQ ID NO:114:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:
AAGACCGGCC GGCGCCAUAG CCGAGAUCCG AGGUGUUG 38

(2) INFORMATION FOR SEQ ID NO:115:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid,
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:
GAGACCGGCC AGCCAAGGCG CUGAGAUCCG AGGUUUCA 38

(2) INFORMATION FOR SEQ ID NO:116:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:
AAGACCGGCC CGGUGUGCAG CCGGAGAUCC GAGACUUGCU G 41

(2) INFORMATION FOR SEQ ID NO:117:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGA 47

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGAACG 50

AUAGACCAAC CGAGAA 66

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 60 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGAGAC 50

CAACCGAGAA 60

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 59 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine

(ix) FEATURE:

(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGAACG 50

AUCCGAGAA 59

(2) INFORMATION FOR SEQ ID NO:121:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:
GGGAAAAGAA GACCGGCCGG CGCCAUAGCC GAGAUCCGAG GUGUUGCCGA 50
GAA 53

(2) INFORMATION FOR SEQ ID NO:122:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: ALL C'S ARE 2'-NH_2 cytosine
(ix) FEATURE:
(D) OTHER INFORMATION: ALL U'S ARE 2'-NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:
GGGAGGACGA UGCGGUAGUA ACGGAUACUG AUCCGAGGUU AUAGUCACUA 50
UAUCAUCCGC UGCGCCAGAC GACUCGCCCG A 81

(2) INFORMATION FOR SEQ ID NO:123:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:
GGGAGCUCAG AAUAAACGCU CAANNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNUUCGACA UGAGGCCCGG AUCCGGC 77

(2) INFORMATION FOR SEQ ID NO:124:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:
CCGAAGCTTA ATACGACTCA CTATAGGGAG CTCAGAATAA ACGCTCAA 48

(2) INFORMATION FOR SEQ ID NO:125:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:
GCCGGATCCG GGCCTCATGT CGAA 24

(2) INFORMATION FOR SEQ ID NO:126:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 2-4, 15, 17-18
(D) OTHER INFORMATION: C at positions 2-4, 15, 17-18 are 2'NH_2 cytosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 5, 8, 11, 23
(D) OTHER INFORMATION: U at positions 5, 8, 11, 23 are 2'NH_2 uracil
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:
ACCCUGAUGG UAGACGCCGG GGUG 24

(2) INFORMATION FOR SEQ ID NO:127:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 2-4, 15, 17-18
(D) OTHER INFORMATION: C at positions 2-4, 15, 17-18 are 2'NH_2 cytosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 5, 8, 11, 23
(D) OTHER INFORMATION: U at positions 5, 8, 11

and 23 are 2'NH_2 uracil
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 12, 14
(D) OTHER INFORMATION: A at positions 12 and 14 are 2'OMe adenosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 13, 16, 19-21, 22, 24
(D) OTHER INFORMATION: G at positions 13, 16, 19-21, 22 and 24 are 2'OMe guanosine
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:
ACCCUGAUGG UAGACGCCGG GGUG 24

(2) INFORMATION FOR SEQ ID NO:128:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 6-8, 19, 21-22
(D) OTHER INFORMATION: C at positions 6-8, 19 and 21-22 are 2'NH_2 cytosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 9, 12, 15, 27
(D) OTHER INFORMATION: U at positions 9, 12, 15 and 27 are 2'NH_2 uracil
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 1-4, 29-33
(D) OTHER INFORMATION: T at positions 1-4 and 29-33 are 2' deoxy phosphorothioate thymidine
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:
TTTTACCCUG AUGGUAGACG CCGGGGUGTT TTT 33

(2) INFORMATION FOR SEQ ID NO:129:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: All A's, C's, G's,

T's are 2' deoxy-nucleotide derivatives
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 1-4, 29-33
(D) OTHER INFORMATION: T at positions 1-4 and 29-33 are phosphorothioate thymidine
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:
TTTTACCCTG ATGGTAGACG TTGGGGTGTT TTT 33

(2) INFORMATION FOR SEQ ID NO:130:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(D) OTHER INFORMATION: All A's, C's, G's, U's are 2'OMe-nucleotide derivatives
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 1-4, 29-33
(D) OTHER INFORMATION: T's at positions 1-4 and 29-33 are 2' deoxy phosphorothioate thymidine
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:
TTTTACCCUG AUGGUAGACG CCGGGGUGTT TTT 33

(2) INFORMATION FOR SEQ ID NO:131:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 6-8, 19, 21-22
(D) OTHER INFORMATION: C at positions 6-8, 19 and 21-22 are 2'NH_2 cytosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 9, 12, 15, 27
(D) OTHER INFORMATION: U at positions 9, 12, 15 and 27 are 2'NH_2 uracil
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 5, 16
(D) OTHER INFORMATION: A at positions 5 and

16 are 2'OMe adenine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 13, 17, 20, 23-26, 28
(D) OTHER INFORMATION: G at positions 13, 17, 20, 23-26 and 28 are 2'OMe guanosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 1-4, 29-33
(D) OTHER INFORMATION: T's at positions 1-4 and 29-33 are 2' deoxy phosphorothioate thymidine
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:
TTTTACCCUG AUGGUAGACG CCGGGGUGTT TTT 33

(2) INFORMATION FOR SEQ ID NO:132:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 6-8, 19, 21-22
(D) OTHER INFORMATION: C at positions 6-8, 19 and 21-22 are 2'NH_2 cytosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 5, 8, 11, 23
(D) OTHER INFORMATION: U at positions 9, 12, 15 and 27 are 2'NH_2 uracil
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 5, 16
(D) OTHER INFORMATION: A at positions 5 and 16 are 2'OMe adenine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 13, 17, 20, 24-26, 28
(D) OTHER INFORMATION: G at positions 13, 17, 20, 24-26 and 28 are 2'OMe guanosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 1-4, 29-33
(D) OTHER INFORMATION: T's at positions 1-4 and 29-33 are 2' deoxy phosphorothioate thymidine
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

TTTTACCCUG AUGGUAGACG CCGGGGUGTT TTT 33

(2) INFORMATION FOR SEQ ID NO:133:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 6-8, 19, 21-22
(D) OTHER INFORMATION: C at positions 6-8, 19 and 21-22 are 2'NH_2 cytosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 9, 12, 15, 27
(D) OTHER INFORMATION: U at positions 9, 12, 15 and 27 are 2'NH_2 uracil
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 5, 16
(D) OTHER INFORMATION: A at positions 5 and 16 are 2'OMe adenine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 13, 17, 20, 23-26, 28
(D) OTHER INFORMATION: G at positions 13, 17, 20, 23-26 and 28 are 2'OMe guanosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 11
(D) OTHER INFORMATION: A at position 11 is phosphorothioate adenine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 1-4, 29-33
(D) OTHER INFORMATION: T's at positions 1-4 and 29-33 are 2' deoxy phosphorothioate thymidine
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:
TTTTACCCUG AUGGUAGACG CCGGGGUGTT TTT 33

(2) INFORMATION FOR SEQ ID NO:134:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 2-4, 15, 17-18

(D) OTHER INFORMATION: C at positions 2-4, 15 and 17-18 are 2'NH_2 cytosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 5, 8, 11, 23

(D) OTHER INFORMATION: U at positions 5, 8, 11 and 23 are 2'NH_2 uracil

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 9

(D) OTHER INFORMATION: G at position 9 is a 2:1 misture of 2'OMe guanosine and 2'OH guanosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 12, 14

(D) OTHER INFORMATION: A at positions 12 and 14 are a 2:1 mixture of 2'OMe adenine and 2'OH adenine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

ACCCUGAUGG UAGACGCCGG GGUG 24

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 2-4, 15, 17-18

(D) OTHER INFORMATION: C at positions 2-4, 15 and 17-18 are 2'NH_2 cytosine

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 5, 8, 11, 23

(D) OTHER INFORMATION: U at positions 5, 8, 11 and 23 are 2'NH_2 uracil

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 1 and 7

(D) OTHER INFORMATION: A at positions 1 and 7 are a 2:1 mixture of 2'OMe adenine and 2'OH adenine

(ix) FEATURE:

(A) NAME/KEY: modified base
(B) LOCATION: 19 and 21
(D) OTHER INFORMATION: G at positions 19 and 21 are a 2:1 mixture of 2'OMe guanosine and 2'OH guanosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:
ACCCUGAUGG UAGACGCCGG GGUG 24

(2) INFORMATION FOR SEQ ID NO:136:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 2-4, 15, 17-18
(D) OTHER INFORMATION: C at positions 2-4, 15 and 17-18 are 2'NH_2 cytosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 5, 8, 11, 23
(D) OTHER INFORMATION: U at positions 5, 8, 11 and 23 are 2'NH_2 uracil
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 6, 20, 24
(D) OTHER INFORMATION: G at positions 6, 20, and 24 are a 2:1 mixture of 2'OMe guanosine and 2'OH guanosine
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:
ACCCUGAUGG UAGACGCCGG GGUG 24

(2) INFORMATION FOR SEQ ID NO:137:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 2-4, 15, 17-18
(D) OTHER INFORMATION: C at positions 2-4, 15 and 17-18 are 2'NH_2 cytosine
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 5, 8, 11, 23

(D) OTHER INFORMATION: U at positions 5, 8, 11 and 23 are 2'NH_2 uracil

(ix) FEATURE:

(A) NAME/KEY: modified base

(B) LOCATION: 10, 13, 16

(D) OTHER INFORMATION: G at positions 10, 13 and 16 are a 2:1 mixture of 2'OMe guanosine and 2'OH guanosine

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

ACCCUGAUGG UAGACGCCGG GGUG 24

(2) INFORMATION FOR SEQ ID NO:138:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

ATCCGCCTGA TTAGCGATAC TACAACGGCG TGGAAGACTA GAGTGCAGCC 50

GAACGCATCT AACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:139:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

ATCCGCCTGA TTAGCGATAC TACGCTACAA GTCCGCTGTG GTAGACAAGA 50

GTGCAGGCAA GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:140:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

ATCCGCCTGA TTAGCGATAC TAGGCCCGTC GAAGNTAGAG CGCAGGGCCC 50

CAAAATACCG ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:141:

ATCCGCCTGA TTAGCGATAC TGTACCATCC ACGGTTTACG TGGACAAGAG 50
GGCCCTGGTA CACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:142:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:142:

ATCCGCCTGA TTAGCGATAC TTCACTACAA GTCCGCCGTG GTAGACAAGA 50
GTGCAGGCAA GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:143:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

ATCCGCCTGA TTAGCGATAC TACCGCTGTG TAGTTCCTTT AGGACTAGAG 50
GGCCGCCTAC ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:144:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

ATCCGCCTGA TTAGCGATAC TTAGGCTTGA CGTCTTCTAG ACTAGAGTGC 50
AGTCAAACCC ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:145:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

ATCCGCCTGA TTAGCGATAC TTGCAGGTCG ACTCTAGAGG ATCCCCGGGT 50
ACCGAGCTCG AACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:146:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 77 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:146:

ATCCGCCTGA TTAGCGATAC TACGGTTTAC GTGGACAAGA GGGCCCTGGT 50
ACACTTGAGC AAAATCACCT GCAGGGG 77

(2) INFORMATION FOR SEQ ID NO:147:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:147:

ATCCGCCTGA TTAGCGATAC TGGTGGACTA GAGGNCAGCA AACGATCCTT 50
GGTTCGCGTC CACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:148:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

ATCCGCCTGA TTAGCGATAC TTCAAGCACT CCCGTCTTCC AGACAAGAGT 50
GCAGGGCCTC TACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:149:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

ATCCGCCTGA TTAGCGATAC TCGTGATGGA CAAGAGGGCC CTATCCACCG 50
GATATCCGTC ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:150:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

ATCCGCCTGA TTAGCGATAC TCAAGCAGTG CCCGTCTTCC AGACAAGAGT 50
GCAGGCCTCT ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:151:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:
ATCCGCCTGA TTAGCGATAC TTGATCCACC GTTTATAGTC CGTGGTAGAC 50
AAGAGTGCAG GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:152:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:
ATCCGCCTGA TTAGCGATAC TAACACACAA GAGGACAGTT ACAGGTAACA 50
TCCGCTCAGG ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:153:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 83 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:
ATCCGCCTGA TTAGCGATAC TAGTGGCGTC TATAGACAAG AGTGCAGCCC 50
GAGTTTCAAC TTGAGCAAAA TCACCTGCAG GGG 83

(2) INFORMATION FOR SEQ ID NO:154:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:
ATCCGCCTGA TTAGCGATAC TCCACAAGAG GGCAGCAAGT GTACAACTAC 50
AGCGTCCGGA CTTGAGCAAA ATCACCTGCA GGGG 84

(2) INFORMATION FOR SEQ ID NO:155:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 79 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:155:

CTACCTACGA TCTGACTAGC GCAGGGCCAC GTCTATTTAG ACTAGAGTGC 50
AGTGGTTCGC TTACTCTCAT GTAGTTCCT 79

(2) INFORMATION FOR SEQ ID NO:156:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:156:

CTACCTACGA TCTGACTAGC ACGGTCCAAA GGTTTCCCAT CCGTGGACTA 50
GAGGGCACGT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:157:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:157:

CTACCTACGA TCTGACTAGC CCGTCGCGTG ACTATAACCA CACGCAGACT 50
AGAGTGCAGG GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:158:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 80 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:158:

CTACCTACGA TCTGACTAGC CCGAATGGGG CTGCGACTGC AGTGGACGTC 50
ACGTCGTTAG CTTACTCTCA TGTAGTTCCT 80

(2) INFORMATION FOR SEQ ID NO:159:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 80 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:159:

CTACCTACGA TCTGACTAGC ACGCAAGAGA GTCNCCGAAT GCAGTCTCAG 50
CCGCTAACAG CTTACTCTCA TGTAGTTCCT 80

(2) INFORMATION FOR SEQ ID NO:160:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:160:

ATCCGCCTGA TTAGCGATAC TCANNNCACT GCAAGCAATT GTGGCCCAAA 50
GGGCTGAGTA CTTGAGCAAA ATCACCTGCA GGGG 84

(2) INFORMATION FOR SEQ ID NO:161:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:161:

ATCCGCCTGA TTAGCGATAC TGCTCGCTTA CAAAAGGGAG CCACTGTAGC 50
CCAGACTGGA CACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:162:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:162:

ATCCGCCTGA TTAGCGATAC TGGTTATGGT GTGGTTCCGA ATGGTGGGCA 50
AAGTAACGCT TACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:163:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:163:

ATCCGCCTGA TTAGCGATAC TGCTTGTNGC TCCGAAGGGG CGCGTATCCA 50
AGGACGGTTC ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:164:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 83 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:164:

ATCCGCCTGA TTAGCGATAC TTATGGAGTG GTTCCGAATG GTGGGCAAAG 50
TAACGCTTAC TTGAGCAAAA TCACCTGCAG GGG 83

(2) INFORMATION FOR SEQ ID NO:165:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:165:

CTACCTACGA TCTGACTAGC TGCNNGCGGG CGGTTCTCCG GATGGGACCA 50
TAAGGCTTTA GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:166:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 80 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:166:

CTACCTACGA TCTGACTAGC ACAAGGGGTC CTGNNGAATG GGGGAATACG 50
CTAGCCGAAG CTTACTCTCA TGTAGTTCCT 80

(2) INFORMATION FOR SEQ ID NO:167:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:167:

CTACCTACGA TCTGACTAGC AACACGAGCA TGTGGGGTCC CTTCCGAATG 50
GGGGGTACAG GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:168:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:168:

CTACCTACGA TCTGACTAGC GAGGCATTAG GTCCGAATGG TAGTAATGCT 50
GTCGTGCCTT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:169:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 80 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:169:

CTACCTACGA TCTGACTAGC CCGAATGGGG CTGCGACTGC AGTGGACGTC 50
ACGTCGTTAG CTTACTCTCA TGTAGTTCCT 80

(2) INFORMATION FOR SEQ ID NO:170:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:170:

CTACCTACGA TCTGACTAGC GAGGAGGTGC GTTGTCCGAA GGGGTCGTTA 50
GTCACCTCGT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:171:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:171:

CTACCTACGA TCTGACTAGC GCAAGGGGTC CTGCCGAATG GGGGAATACG 50
CTAGCCGAAA GCTTACTCTC ATGTAGTTCC T 81

(2) INFORMATION FOR SEQ ID NO:172:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:172:

CTACCTACGA TCTGACTAGC ATCCTTCCGA ATGGGGGAAA TGGCGNCCCA 50
GCTTACTCTC ATGTAGTTCC T 71

(2) INFORMATION FOR SEQ ID NO:173:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 82 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:173:

CTACCTACGA TCTGACTAGC ACGCAAGAGA GGTCNCCGAA TGGCAGTCTC 50

AGCCGCTAAC AGCTTACTCT CATGTAGTTC CT 82

(2) INFORMATION FOR SEQ ID NO:174:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:174:
CTACCTACGA TCTGACTAGC CACGATAATC CTCCGAAAGC GTTGTCCGAA 50
TGGGTCGTTA GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:175:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:175:
ATCCGCCTGA TTAGCGATAC TTATCACCCC CACTGGATAG AGCCGCAGCG 50
TGCCCCTACT ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:176:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:176:
ATCCGCCTGA TTAGCGATAC TGCCCACTGC ATAGAGGGAC GGTTGTTTCC 50
GCCCGGTGTT TACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:177:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:177:
CTACCTACGA TCTGACTAGC GTGAAGGAGC CCCAACTGGA TAGAAGCCTT 50
AAGGCGGTGT GCTTACTCTC ATGTAGTTCC T 81

(2) INFORMATION FOR SEQ ID NO:178:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:178:

CTACCTACGA TCTGACTAGC CCACCGCAGA GTGTTACACC CCATAGGAGA 50
AGTCCGGATG GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:179:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 87 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:179:

CTACCTACGA TCTGACTAGC CCACTGCATA GAGAGTCGCA AGACACGGTG 50
CTTTATTCNC CGCTTAGCTT ACTCTCATGT AGTTCCT 87

(2) INFORMATION FOR SEQ ID NO:180:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:180:

CTACCTACGA TCTGACTAGC TGCCCCACTG GATAGAGTAG GAGGCCTAGC 50
CGACACGGTG CTTAGCTTAC TCTCATGTAG TTCCT 85

(2) INFORMATION FOR SEQ ID NO:181:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:181:

CTACCTACGA TCTGACTAGC CGAGGTCCCC CACTGGATAG AGTTGTTGAA 50
ACAACGGTGC GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:182:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:182:

CTACCTACGA TCTGACTAGC AACACTTCCC CACTGGATAG AGGCCTTTCG 50
CAGAGCCGGT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:183:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:183:

CTACCTACGA TCTGACTAGC CCACTGCATA GAGAACTGGA TCGACGGTCC 50
AAAGTTCGGT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:184:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 89 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:184:

CTACCTACGA TCTGACTAGC CCACTGCATA GAGATACTGG ATTCGACNNN 50
CCAAAGTTTC GGTGCTTAGC TTACTCTCAT GTAGTTCCT 89

(2) INFORMATION FOR SEQ ID NO:185:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:185:

CTACCTACGA TCTGACTAGC CCACTGCAGA GAGTCAACCT TACGANGCCA 50
AGGTTGCGGT GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:186:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:186:

ATCCGCCTGA TTAGCGATAC TTCTGCGAGA GACCTACTGG AACGTTTTGT 50
GATATTCACA ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:187:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:187:

ATCCGCCTGA TTAGCGATAC TATACACCCG GCGGGCCTAC CGGATCGTTG 50
ATTTCTCTCC ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:188:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:188:

ATCCGCCTGA TTAGCGATAC TACGCCCCCT GAGACCTACC GGAATNTTNT 50
CGCTAGGCCT AACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:189:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:189:

ATCCGCCTGA TTAGCGATAC TGGGCATCTA ACCCAGACCT ACCGGAACGT 50
TATCGCTTGT GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:190:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 83 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:190:

ATCCGCCTGA TTAGCGATAC TGGTGTGAAC CAGACCTACN GGAACGTTAT 50
CGCTTGTGAC TTGAGCAAAA TCACCTGCAG GGG 83

(2) INFORMATION FOR SEQ ID NO:191:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:191:

ATCCGCCTGA TTAGCGATAC TCATCAGTAT TATATAACGG GAACCAACGG 50
CAAATGCTGA CACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:192:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:192:

ATCCGCCTGA TTAGCGATAC TTCCNNGGGA GAATAGGGTT AGTCGGAGAA 50
GTTAATCGCT ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:193:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:193:

ATCCGCCTGA TTAGCGATAC TCGGGAACGT GTGGTTACNC GGCCTACTGG 50
ATTGTTTCCT GACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:194:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:194:

ATCCGCCTGA TTAGCGATAC TGGTAGGTCC GGTGTGAAAG AGGTTCGCAT 50
CAGGTAACTT GAGCAAAATC ACCTGCAGGG G 81

(2) INFORMATION FOR SEQ ID NO:195:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 85 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:195:

ATCCGCCTGA TTAGCGATAC TCCTCAGGCA ACATAGTTGA GCATCGTATC 50
GATCCTGGAG ACTTGAGCAA AATCACCTGC AGGGG 85

(2) INFORMATION FOR SEQ ID NO:196:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 84 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:196:

ATCCGCCTGA TTAGCGATAC TTTGGCTTGA GTCCCGGGAC GCACTGTTGA 50

CAGTGGAGTA CTTGAGCAAA ATCACCTGCA GGGG 84

(2) INFORMATION FOR SEQ ID NO:197:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:197:
ATCCGCCTGA TTAGCGATAC TCAGCAGGTT AGTATAACGG GAACCAACGG 50
CAAATGCTGA CACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:198:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:198:
CTACCTACGA TCTGACTAGC GCAAGGGCAT CTCGGAATCG GTTAATCTGA 50
CTTGCAATAC GCTTAGCTTA CTCTCATGTA GTTCCT 86

(2) INFORMATION FOR SEQ ID NO:199:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 75 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:199:
CTACCTACGA TCTGACTAGC GATCCACGAA GAAGCTTACT CTCATGTAGT 50
TCCAGCTTAC TCTCATGTAG TTCCT 75

(2) INFORMATION FOR SEQ ID NO:200:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:200:
GTACCATCCA CGGTTTACGT GGACAAGAGG GCCCTGGTAC 40

(2) INFORMATION FOR SEQ ID NO:201:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:201:

GTAGTTCCTT TAGGACTAGA GGGCCGCCTA C 31

(2) INFORMATION FOR SEQ ID NO:202:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:202:

TGGACTAGAG GNCAGCAAAC GATCCTTGGT TCGCGTCC 38

(2) INFORMATION FOR SEQ ID NO:203:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:203:

CCCGTCTTCC AGACAAGAGT GCAGGG 26

(2) INFORMATION FOR SEQ ID NO:204:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:204:

AGGGCCACGT CTATTTAGAC TAGAGTGCAG TGGTTC 36

(2) INFORMATION FOR SEQ ID NO:205:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:205:

GGAGGTGCGT TGTCCGAAGG GGTCGTTAGT CACCTC 36

(2) INFORMATION FOR SEQ ID NO:206:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:206:
GCAAGGGGTC CTGCCGAATG GGGGAATACG CTAGCCGAAA 40

(2) INFORMATION FOR SEQ ID NO:207:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:207:
CGAGGTCCCC CACTGGATAG AGTTGTTGAA ACAACGGTGC GCTTA 45

(2) INFORMATION FOR SEQ ID NO:208:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:208:
AACACTTCCC CACTGGATAG AGGCCTTTCG CAGAGCCGGT GCTTA 45

(2) INFORMATION FOR SEQ ID NO:209:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:209:
GGGCATCTAA CCCAGACCTA CCGGAACGTT ATCGCTTGTG 40

(2) INFORMATION FOR SEQ ID NO:210:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:210:
AGACAAGAGT GCAGG 15

(2) INFORMATION FOR SEQ ID NO:211:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:211:

GGACTAGAGG GCAGT 15

(2) INFORMATION FOR SEQ ID NO:212:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:212:
CTCCGAATGG GGGNAAAG 18

(2) INFORMATION FOR SEQ ID NO:213:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:213:
CCCCACTGGA TAGAG 15

(2) INFORMATION FOR SEQ ID NO:214:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:214:
CCCCACTGCA TAGAG 15

(2) INFORMATION FOR SEQ ID NO:215:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:215:
AGACCTACCG GAACGTT 17

(2) INFORMATION FOR SEQ ID NO:216:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:216:
ATCCGCCTGA TTAGCGATAC TNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 50

NNNNNNNNNN NACTTGAGCA AAATCACCTG CAGGGG 86

(2) INFORMATION FOR SEQ ID NO:217:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 1
(D) OTHER INFORMATION: N equals 3 biotin
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:217:
NCCCCTGCAG GTGATTTTGC TCAAGT 26

(2) INFORMATION FOR SEQ ID NO:218:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:218:
ATCCGCCTGA TTAGCGATAC T 21

(2) INFORMATION FOR SEQ ID NO:219:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 81 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:219:
CTACCTACGA TCTGACTAGC NNNNNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN GCTTACTCTC ATGTAGTTCC T 81

(2) INFORMATION FOR SEQ ID NO:220:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ix) FEATURE:
(A) NAME/KEY: modified base
(B) LOCATION: 2 and 4
(D) OTHER INFORMATION: N is biotin
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:220:
ANANAGGAAC TACATGAGAG TAAGC 25

(2) INFORMATION FOR SEQ ID NO:221:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:221:
CTACCTACGA TCTGACTAGC 20

(2) INFORMATION FOR SEQ ID NO:222:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: DNA

(xi SEQUENCE DESCRIPTION: SEQ ID NO:222:
GGGAGACAAG AATAAACGCT CAANNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN NNNNNNNNNN NNNTTCGACA GGAGGCTCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:223:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:223:
TAATACGACT CACTATAGGG AGACAAGAAT AAACGCTCAA 40

(2) INFORMATION FOR SEQ ID NO:224:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:224:
GCCTGTTGTG AGCCTCCTGT CGAA 24

(2) INFORMATION FOR SEQ ID NO:225:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:225:
GGGAGACAAG AAUAAACGCU CAANNNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN NNNNNNNNNN NNNUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:226:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:226:
GCCGGATCCG CCTGTTGTGA GCCTCCTGTC GAA 33

(2) INFORMATION FOR SEQ ID NO:227:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:227:
CCGAAGCTTA ATACGACTCA CTATAGGGAG ACAAGAATAA ACGCTCAA 48

(2) INFORMATION FOR SEQ ID NO:228:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:228:
TAATACGACT CACTATA 17

(2) INFORMATION FOR SEQ ID NO:229:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:229:
GGGAGACAAG AAUAAACGCU CAAUUGUGAU CUACCGUUAC CUGACGGACG 50
UGUUUUUACA CCAACGAACC UGGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:230:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:230:
GGGAGACAAG AAUAAACGCU CAAUGAACCU CUGAGUUCUU CAUAGAACUG 50
AUAUCUUCAA GAAGGGGUAG AUGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:231:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:231:
GGGAGACAAG AAUAAACGCU CAACGUCUUC UACAGGGAAC CGUGGUGCAU 50
CUGUGAAGUU GUAGAUUCCU AGUUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:232:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:232:
GGGAGACAAG AAUAAACGCU CAAGGGUUGA UUACCAAAUC GUAACCUGUA 50
CCCUGCCUAC ACUAGGACAA CGGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:233:

(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:233:
GGGAGACAAG AAUAAACGCU CAACUAACCG AAGCUCUGAG AAAUAGUUUA 50
UCCAGUGAAU GAAUCCUGAU GGGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:234:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:234:
GGGAGACAAG AAUAAACGCU CAACGAGCUU UUAGUAAGAC UCAUGCCGAG 50
AAAUCGGUCG UGAUGCUGUC GAGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:235:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:235:
GGGAGACAAG AAUAAACGCU CAAGCCCGGG AAUUGCAUGU UGUGCGUGCC 50
GGGAGUCCAA GUCAGCAUCC UCAUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:236:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:236:
GGGAGACAAG AAUAAACGCU CAAGGGUUGA UUACCAAAUC GUAACCUGUA 50
CCCUGCCUAC CCUAGGACAA CGAUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:237:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:237:
GGGAGACAGA AUAAACGCUC AAAUCCCACU CGGUCGUGAC CUGACGUGAA 50
AACGGAUAGG AUCGCACGUC AAUUCGACAG GAGGCUCACA ACAGGC 96

(2) INFORMATION FOR SEQ ID NO:238:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:238:
GGGAGACAAG AAUAAACGCU CAAGUGUCCC UUGUCACCUG GGACUGGGCC 50
GUUUGAACUG ACAUUCUAUA CGAUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:239:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:239:
GGGAGACAAG AAUAAACGCU CAAGGCCAAC GUCCGAGUUG GUUUCCCAUA 50
GCUGAGCACA GGACGGCUUC UGCUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:240:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:240:
GGGAGACAAG AAUAAACGCU CAACCCGGAU GGUGAGCCCU GUCGGAGAUU 50

GGCACGAGGG UAAAGGGUAG GGAUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:241:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:241:
GGGAGACAAG AAUAAACGCU CAACUGUUGC UAAGUAGAAG UCAUAUUCUG 50
CGAUGGUUAA AGAUAACCCA GCCUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:242:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 95 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:242:
GGGAGACAAG AAUAAACGCU CAAUCUCGUA UCAGCACACC GGUACAAAGA 50
GGAUGCAAAU NCGCCUGUGA CUUCGACAGG AGGCUCACAA CAGGC 95

(2) INFORMATION FOR SEQ ID NO:243:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 100 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:243:
GGGAGACAAG AAUAAACGCU CAACGUCUUC UACAGGGAAC CGUGGUGCAU 50
CUGUGAAGUU GUUGUAGAUU CCUAGAUUCG ACAGGAGGCU CACAACAGGC 100

(2) INFORMATION FOR SEQ ID NO:244:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:244:

GGGAGACAAG AAUAAACGCU CAAUGUCUUG GCGUCCACGU CGUAGUGUGU 50
GGGGGGAAAA GAGGAGGGUG CACUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:245:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:245:

GGGAGACAAG AAUAAACGCU CAAUUGUUAG CCUGGUGAAG CAUGUACUGC 50
GAAGUGGAGG GAAUGUGGAA GGGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:246:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:246:

GGGAGACAAG AAUAAACGCU CAAGCUCCCC UAGCCGACUU CCUGUUGGAC 50
GAGGGUGGUA CCGUGGGAGG AUGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:247:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:247:

GGGAGACAAG AAUAAACGCU CAACGAGCCU UUAGUAAGAC UCAUGCCGAG 50
AAAUCGGUCG UGAUGCUGUC GAGUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:248:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:248:

GGGAGACAAG AAUAAACGCU CAAUUCGUGA GGGCGGUGUG GGAGGCAAGC 50
GGUACGAGCG UACUGUCUGG GCCUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:249:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:249:

GGGAAAAGCG AATCATACAC AAGANNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN NNNNNNNNNN NNNNGCTCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:250:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:250:

TAATACGACT CACTATAGGG AAAAGCGAAT CATACACAAG A 41

(2) INFORMATION FOR SEQ ID NO:251:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:251:

TTCTCGGTTG GTCTCTGGCG GAGC 24

(2) INFORMATION FOR SEQ ID NO:252:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:252:

GGGAAAAGCG AAUCAUACAC AAGANNNNNN NNNNNNNNNN NNNNNNNNNN 50
NNNNNNNNNN NNNNNNNNNN NNNNGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:253:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:253:

CGCGGATCCT AATACGACTC ACTATAGGGA AAAGCGAATC ATACACAAGA 50

(2) INFORMATION FOR SEQ ID NO:254:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:254:

GGCGAATTCT TCTCGGTTGG TCTCTGGCGG AGC 33

(2) INFORMATION FOR SEQ ID NO:255:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULARY TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:255:

GGGAAAAGCG AAUCAUACAC AAGAUGUGAU GGGUGUGUUU UGGUUAGCUG 50
UGGAGGGCCA UGUGGGCUGG ACGGCUCCGC CAGAGACCAA CCGAGAA 97

(2) INFORMATION FOR SEQ ID NO:256:

(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:256:

GGGAAAAGCG AAUCAUACAC AAGAGGUGUG UGUGCGCCGC GAGGUGUCUG 50
AGGAGAGAGG UGGCAGUGGA GGGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:257:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:257:

GGGAAAAGCG AAUCAUACAC AAGAGGAGGG UGUGCGGACG GGAGCGUGUA 50
GUGAGGCUUU UCAGGCGUUG GACGGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:258:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:258:

GGGAAAAGCG AAUCAUACAC AAGAUGUGAU GGGUGUGAUU UUGGUUAGCU 50
GUGGAGGGUU AUGUGGGUCG GACGGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:259:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:259:

GGGAAAAGCG AAUCAUACAC AAGAUGUGGG CUUCCUGAGG GGUAGGAACU 50
CUGAAGUCAU GGUUCGUGGU AAGCGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:260:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:260:
GGGAAAAGCG AAUCAUACAC AAGAGGAGGG AAUGUGGAAG GGUUUGUGGU 50
GUUUCGCAAA UGCCGCAUGG ACGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:261:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:261:
GGGAAAAGCG AAUCAUACAC AAGAGGGGGG AGAGGCGUGG ACGAUGUUGU 50
GGUUAUGCUG UCGGUUUUGG CUUGGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:262:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:262:
GGGAAAAGCG AAUCAUACAC AAGAGGGAUG GAUCGGUGAG ACGAGCAGUG 50
GAGUGGUGAG GUGUGGUGUC ACGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:263:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:263:
GGGAAAAGCG AAUCAUACAC AAGACGUUCG GUGGUGGACA GGGUAAUGUG 50
GAGGGACCGG GUGAUUGUGU AUGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:264:
(i) SEQUENCE CHARACTERIZATION:

(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:264:

GGGAAAAGCG AAUCAUACAC AAGAUGGAGG GUGGCGGGAG AAGUUUGAGG 50
UCGGGGUCGU AUGAUGUGCG CUAGGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:265:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:265:

GGGAAAAGCG AAUCAUACAC AAGAUCACGA CGGAGGGCGG AUGAAGGGGG 50
GAAGGUCGUG AGUCCAUGCC GUGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:266:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:266:

GGGAAAAGCG AAUCAUACAC AAGAGGAUGG GCGUGAAUGG AGGGCAAUGU 50
GUGGGUUUGU UGAGCCAGUU GGUGCUCCGC CAGAGACCAA CCGAGAA 97

(2) INFORMATION FOR SEQ ID NO:267:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:267:

GGGAAAAGCG AAUCAUACAC AAGAACGGGU CCCUAAGACG UCGUUUCGAG 50
AUGUGUGUUU CGGAAAGAGU AGCGGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:268:

(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:268:
GGGAAAAGCG AAUCAUACAC AAGAACAGCG AUUUGUGUGC UCGUCGGCUG 50
UUUGCAGAUG UAUGGAACCA UGCAGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:269:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:269:
GGGAAAAGCG AAUCAUACAC AAGAGGGCUU GGGGUGGGGU CUUGCAGACG 50
GAUAUGGUAA GCUGCUGCUG GCUGCUCCGC CAGAGACCAA CCGAGAA 97

(2) INFORMATION FOR SEQ ID NO:270:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:270:
GGGAAAAGCG AAUCAUACAC AAGAAUUGGG AACGUGGCGC AUCGGGAGUG 50
UAUGGUGGCU UGUGGGCGGG UUAGCUCCGC CAGAGACCAA CCGAGAA 97

(2) INFORMATION FOR SEQ ID NO:271:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:271:
GGGAAAAGCG AAUCAUACAC AAGAAGCCAG GUCUUCAAUG GUAGUUGUUG 50
AUUGGUGGUG CUUUGCAGAU GUAUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:272:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:272:
GGGAAAAGCG AAUCAUACAC AAGAUCGGUU AUGGAGAGGC GGUAGAGAGC 50
GAGUAGCAAG UAACGGUGCA AAAGGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:273:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:273:
GGGAAAAGCG AAUCAUACAC AAGACCGCAC UUCGAUUGCA UUGUCGAGGA 50
GAGCGGGUGC AGAUGUACGG GUUUGGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:274:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 99 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:274:
GGGAAAAGCG AAUCAUACAC AAGAAGACUU GGUACGCGUG GUUAGCUAGG 50
AGGGGGGGAG AAGAUAAGGC GAUAGGCUCC GCCAGAGACC AACCGAGAA 99

(2) INFORMATION FOR SEQ ID NO:275:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:275:
GGGAAAAGCG AAUCAUACAC AAGAGAGUUU UGCUUCGACA UGGGGUUAGC 50

UGUGGAGCAG GGUGACCUGG GACCGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:276:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:276:
GGGAAAAGCG AAUCAUACAC AAGAUGGAGU GGGUAGGCGU GGGAGGUAUG 50
GCAUCUUGUG UGUGCCAAGA UGGUGCUCCG CCAGAGACCA ACCGAGAA 98

(2) INFORMATION FOR SEQ ID NO:277:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:277:
GGGUCAUACA CAAGAACAGU GAUUUGUGUG CUCGUCGGCU GUUUGCGAGU 50
UAU 53

(2) INFORMATION FOR SEQ ID NO:278:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 63 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:278:
GGGAUACACA AGAAGCCAGG UCUUCAAUGG UAGUUGUUGA UUGGUGGUGC 50
UUUGCAGAUG UAU 63

(2) INFORMATION FOR SEQ ID NO:279:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:279:

GGGAGAGGCG GUAGAGAGCG AGUAGCAAGU AACGGUGCAA AAGGCUCCGC 50
C 51

(2) INFORMATION FOR SEQ ID NO:280:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:280:

GGGUUUGGUU AGCUGUGGAG GGCCAUGAGG GCUGGA 36

(2) INFORMATION FOR SEQ ID NO:281:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 65 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:281:

GGGCAUACAC AAGACGUUCG GUGGUGGACA GGGUAAUGUG GAGGGACCGG 50
GUGAUUGUGU AUGUG 65

(2) INFORMATION FOR SEQ ID NO:282:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 58 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:282:

GGAUCACGAC GGAGGGCGGA UGAAGGGGGG AAGGUCGUGA GUCCAUGCCG 50
UGUGCUCC 58

(2) INFORMATION FOR SEQ ID NO:283:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:283:
GGGCGUGGUG CAUCUGUGCC C 21

(2) INFORMATION FOR SEQ ID NO:284:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:284:
GGGAGCUACA GGGAACCGUG GUGCAUCUG 29

(2) INFORMATION FOR SEQ ID NO:285:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:285:
GGGGUAUCAG CACACCGGUA CAAAGAGGAU GC 32

(2) INFORMATION FOR SEQ ID NO:286:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 96 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:286:
GGGAAAAGCG AAUCAUACAC AAGAUCGGUU AUGGAGAGGC GGUAGAGAGC 50
GAGUAAAGUA ACGGUGCAAA AGGCUCCGCC AGAGACCAAC CGAGAA 96

(2) INFORMATION FOR SEQ ID NO:287:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:287:

GGGAGACAAG AAUAAACGCU CAACGUCUUC UACAGGGAAC CGUGGUCCAU 50
CUGUGAAGUU GUAGAUUCCU AGUUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:288:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 97 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:288:
GGGAGACAAG AAUAAACGCU CAACGUCUUC UACAGGGAAC CGUGGCGCGC 50
CUGUGAAGUU GUAGAUUCCU AGUUUCGACA GGAGGCUCAC AACAGGC 97

(2) INFORMATION FOR SEQ ID NO:289:
(i) SEQUENCE CHARACTERIZATION:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) MOLECULAR TYPE: RNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:289:
RNYAGNNNNN NRGNRNCANR NRRNGNUGNA 30

We Claim:

1. A method for identifying nucleic acid ligands and nucleic acid ligand sequences to secretory phospholipase A2 ($sPLA_2$) comprising:

a) contacting a candidate mixture of nucleic acids with $sPLA_2$, wherein nucleic acids having an increased affinity to the $sPLA_2$ relative to the candidate mixture may be partitioned from the remainder of the candidate mixture;

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to $sPLA_2$, whereby nucleic acid ligands of $sPLA_2$ may be identified.

2. The method of Claim 1 further comprising:

d) repeating steps a), b) and c).

3. The method of Claim 1 wherein said candidate mixture is comprised of single-stranded nucleic acids.

4. The method of Claim 3 wherein said single-stranded nucleic acids are ribonucleic acids.

5. The method of Claim 3 wherein said single-stranded nucleic acids are deoxyribonucleic acids.

6. A nucleic acid ligand to $sPLA_2$ identified according to the method of Claim 1.

7. A purified and isolated non-naturally occurring nucleic acid ligand to secretory phospholipase A_2 ($sPLA_2$).

8. The nucleic acid ligand of Claim 7 which is a ribonucleic acid ligand.

9. The RNA ligand of Claim 8 wherein said RNA ligand is selected from the group consisting of the nucleotide sequences set forth in Table 2.

10. The RNA ligand of Claim 9 wherein said ligand is selected from the group consisting of SEQ ID NOS: 10-122.

11. A purified and isolated non-naturally occurring RNA ligand to $sPLA_2$, wherein said ligand is substantially homologous to and has substantially the same ability to bind $sPLA_2$ as a ligand selected from the group consisting of the sequences set forth in Table 2.

12. A purified and isolated non-naturally occurring RNA ligand to $sPLA_2$, wherin said ligand has substantially the same structure and the same ability to bind $sPLA_2$ as a ligand selected from the group consisting of the sequences set forth in Table 2.

13. A purified and isolated non-naturally occuring RNA ligand to $sPLA_2$ comprising the sequences selected from the group consisting of SEQ ID NOS: 97-100.

14. The ligand of Claim 7 wherein said ligand has been chemically modified at the sugar and/or phosphate and/or base.

15. A purified and isolated non-naturally occurring nucleic acid ligand to VEGF, wherein said nucleic acid ligand is deoxyribonucleic acid.

16. The DNA ligand of Claim 15 wherein said ligand is selected from the group consisting of the sequences set forth in Table 11.

17. The DNA ligand of Claim 15 wherein said ligand is substantially homologous to and has substantially the same ability to bind VEGF as a ligand selected from the group consisting of the sequences set forth in Table 11.

18. The DNA ligand of Claim 15 wherein said ligand has substantially the same structure and substantially the same ability to bind VEGF as a ligand selected from the group consisting of the sequences set forth in Table 11.

19. A purified and isolated non-naturally occurring nucleic acid ligand to VEGF comprising modified nucleic acids.

20. The nucleic acid ligand of claim 19 wherein said modified nucleic acid is selected from the group consisting of 2'-NH_2 nucleosides, 2'-OMe nucleosides, and phosphorothioate nucleotides.

21. The nucleic acid ligand of claim 20 comprising multiple modified nucleic acids.

22. The nucleic acid ligand of claim 21 wherein said nucleic acid ligand is selected from the group consisting of the sequences set forth in Table 7.

23. A method of identifying nucleic acid ligands and nucleic acid ligand sequences to HIV-1 GAG, comprising:

a) contacting the candidate mixture of nucleic acids with HIV-1 GAG, wherein nucleic acids having an increased affinity to HIV-1 GAG relative to the candidate mixture may be partitioned from the remainder of the candidate mixture; and

b) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; and

c) amplifying the increased affinity nucleic acids to yield a mixture of nucleic acids enriched for nucleic acid sequences with relatively higher affinity and specificity for binding to HIV-1 GAG, whereby nucleic acid ligands of HIV-1 GAG may be identified.

24. The method of claim 1 further comprising:

d) repeating steps a), b), and c).

25. The method of claim 23 wherein said candidate mixture of nucleic acids is comprised of single stranded nucleic acids.

26. The method of claim 25 wherein said single stranded nucleic acids are ribonucleic acids.

27. The method of claim 26 wherein said nucleic acids are modified nucleic acids.

28. The method of claim 27 wherein said nucleic acids are 2'-amino (2'-NH_2) modified ribonucleic acids.

29. The method of claim 27 wherein said nucleic acids are 2'-fluoro (2'-F) modified ribonucleic acids.

30. A nucleic acid ligand to HIV-1 GAG identified according to the method of claim 23.

31. A purified and isolated non-naturally occurring RNA ligand to HIV-1 GAG wherein said ligand is selected from the group consisting of the sequences set forth in Tables 13, 15, 18, 19, and 20.

32. A purified and isolated non-naturally occurring RNA ligand to HIV-1 GAG wherein said ligand is substantially homologous to and has substantially the same ability to bind HIV-1 GAG as a ligand selected from the group consisting of the sequences set forth in Tables 13, 15, 18, 19, and 20.

33. A purified and isolated non-naturally occurring RNA ligand to HIV-1 GAG wherein said ligand has substantially the same structure and substantially the same ability to bind HIV-1 GAG as a ligand selected from the group consisting of the sequences set forth in Tables 13, 15, 18, 19, and 20.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02942

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 21/02, 21/04; C12P 19/34; C12Q 1/68
US CL :435/6, 91.2; 536/22.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 91.2; 536/22.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

935/77, 78

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,270,163 A (L. GOLD ET AL.) 14 December 1993, column 5, lines 32-49; Figure 2; columns 44-45, Example 4.	1-8, 11, 12, 14
Y	WO 91/19813 A1 (THE UNIVERSITY OF COLORADO FOUNDATION, INC.) 26 December 1991, page 9, lines 13-32; Figure 2; pages 82-84, Example 4.	1-8, 11, 12, 14
Y	KRAMER et al. Structure and properties of a human non-pancreatic phospholipase A2. The Journal of Biological Chemistry. 05 April 1989, Vol. 264, No. 10, pages 5768-5775, especially page 5768, paragraph 2.	1-8, 11, 12, 14

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 01 JUNE 1996	Date of mailing of the international search report 26 JUN 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer STEPHANIE W. ZITOMER, Ph.D. Telephone No. 703-308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/02942

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-14, drawn to a first process of identifying nucleic acid ligands to sPLA2 and a first product, nucleic acid ligands thereby identified;
Group II, claim(s) 15-22, drawn to a second product, nucleic acid ligands to VEGF;
Group III, claim(s) 23-29, drawn to a second process of identifying nucleic acid ligands to HIV-1 GAG;
Group IV, claims 30-33, drawn to a third product, nucleic acids to HIV-1 GAG.
The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the first and second processes differ in the target for which the ligands are identified and the first, second and third products are ligands to distinct targets.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/18173

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68 G01N27/00 G01N33/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 29199 A (BIO MERIEUX ;GARNIER FRANCIS (FR)) 2 November 1995 (1995-11-02) page 4, line 22 - line 26; example 6	1-5, 9-13, 15-28, 39,41, 52-61, 63,65-70
A	WO 99 31275 A (NEXSTAR PHARMACEUTICALS INC ;DROLET DANIEL (US); CREIGHTON STEVE ()) 24 June 1999 (1999-06-24)	
A	US 5 003 050 A (KIEL JOHNATHAN L ET AL) 26 March 1991 (1991-03-26) cited in the application	

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 November 2001	06/12/2001

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/18173

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 27604 A (NEXSTAR PHARMACEUTICALS INC ;PARMA DAVID (US); GOLD LARRY (US); JA) 12 September 1996 (1996-09-12) the whole document	44-46, 62,71,73
X	KIEL J L ET AL: "Pulsed microwave induced light, sound, and electrical discharge enhanced by a biopolymer." BIOELECTROMAGNETICS, vol. 20, no. 4, 1999, pages 216-223, XP001041588 the whole document	47-51